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Research Article

Avocado Branch Canker Disease Caused by *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* in Taiwan

Yu-Ping Liang[1], Chao-Jung Wu[1], Hui-Wen Tsai[2], and Hui-Fang Ni[3,*]

Abstract

Liang, Y. P., C. J. Wu, H. W. Tsai, and H. F. Ni. 2021. Avocado branch canker disease caused by *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* in Taiwan. J. Taiwan Agric. Res. 70(2):81–97.

Avocado branch canker disease has been reported in many avocado-growing countries, and is associated with several species in the Botryosphaeriaceae family. In Taiwan, the disease is widely distributed in the main avocado-producing regions, but the pathogens were still unidentified. Thirteen fungal isolates were obtained from necrotic avocado woody tissues from seven orchards located in different regions of Taiwan. Based on morphology and phylogenetic analyses of the internal transcribed spacer sequence and partial sequence of the translation elongation factor 1-α gene, the isolates were identified as either *Lasiodiplodia theobromae* or *Lasiodiplodia pseudotheobromae*. The optimal temperature for mycelial growth for both species was around 30℃. Both species were pathogenic to avocado stems and fruits, causing a whitish exudate at the inoculation site and necrosis on the stem, and black lesions on the fruit. To our knowledge, this is the first report characterizing avocado branch canker in Taiwan, which is different from temperate regions in major pathogenic species. The information obtained in this study will be helpful for understanding the epidemiology of the pathogens and establishing effective disease management strategies in avocado.

Key words: Avocado, Branch canker, *Lasiodiplodia theobromae*, *Lasiodiplodia pseudotheobromae*, Taiwan.

INTRODUCTION

Avocado (*Persea americana* Mill.) is cultivated worldwide in tropical and subtropical regions. It was introduced to Taiwan in the twentieth century, and production has gradually increased due to an increasing consumer demand for avocado in recent years. The major avocado production areas are concentrated in the southern cities of Taiwan, including Tainan and Chiayi. In 2019, the planted acreage was 930 hectares with an annual yield of 10,366 Mg (http://agrstat.coa.gov.tw/sdweb/public/inquiry/InquireAdvance.aspx). Instead of ‘Hass’, which is the most commercially popular avocado worldwide, the major cultivars planted in Taiwan are ‘Hall’, ‘Choquette’, and local cultivars derived mostly from open-pollinated West Indian varieties, such as ‘Hung Shin Yuan’, ‘Tainung No.1 Tasty Red’, ‘Tainung No.2 Green Gold’,

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'Zhongpu Green Skin', 'CAES 2', 'CAES 3', 'CAES 4', and 'Changan'. This is because the West Indian race and its hybrids with the Guatemalan race perform better in tropical areas (Ghosh 2000). However, most orchards are small (less than 2 hectares), and planted with a variety of cultivars with different flower types and harvest seasons ranging from June to February.

Avocado branch cankers have been reported in many avocado-growing countries, including the United States (McDonald & Eskalen 2011), Chile (Valencia *et al.* 2019), Italy (Guarnaccia *et al.* 2016), and Spain (Zea-Bonilla *et al.* 2007; Arjona-Girona *et al.* 2019). Previously published studies indicated that avocado branch canker could occur on twigs, branches, or trunks of avocado (McDonald & Eskalen 2011). Cankers are often associated with wounds from pruning, frost damage, mechanical damage, split bark from wind damage, and grafting. In addition, the incidence and severity of disease is greater in trees suffering from drought stress, nutrient deficiencies, waterlogging, temperature extremes, or damage by insects or other pathogens (Dann *et al.* 2013). The typical symptoms include dark, cracked, or sunken bark, which often exudes a reddish sap that dries to a whitish-beige powder; underneath the canker, the wood is reddish brown or brown (McDonald & Eskalen 2011; Auger *et al.* 2013; Dann *et al.* 2013). A cross section of a cankered branch might show a characteristic wedge-shaped discoloration extending to the xylem (McDonald & Eskalen 2011). Pathogen colonization can affect water and nutrient transport, and weaken the woody tissue, causing the affected branch to wilt rapidly (McDonald & Eskalen 2011; Auger *et al.* 2013; Eskalen *et al.* 2013).

Pathogens associated with avocado branch canker include *Neofusicoccum austral*, *Neofusicoccum luteum*, *Neofusicoccum parvum*, *Neofusicoccum nonquaesitum*, *Fusicoccum aesculi*, *Dothiorella iberica*, *Diaporthe foeniculacea*, *Diaporthe sterilis*, *Diplodia mutila*, *Diplodia pseudoseriata*, *Diplodia seriata* (McDonald & Eskalen 2011; Eskalen *et al.* 2013; Guarnaccia *et al.* 2016), and *Neocosmospora perseae* (Guarnaccia *et al.* 2018). Most of the reported branch canker pathogens are species in the Botryosphaeriaceae family, members of which are associated with a wide variety of woody hosts (Slippers & Wingfield 2007). On avocado, they are also the casual agents of stem-end rot on fruits (Twizeyimana *et al.* 2013; Guarnaccia *et al.* 2016; Valencia *et al.* 2019). In Taiwan, the major species causing avocado stem-end rot are *Lasiodiplodia theobromae*, *N. parvum*, *Neofusicoccum mangiferae*, and *F. aesculi* (Ni *et al.* 2011).

Avocado branch canker is a long-standing problem in Taiwan, but the pathogens have not yet been identified. Therefore, to provide information for establishing effective disease management strategies, the objectives of this study were to identify and characterize the pathogens associated with avocado branch canker in Taiwan by morphological examination and molecular analysis, and evaluate their pathogenicity.

MATERIALS AND METHODS

Field sampling and fungal isolation

Sampling was conducted in seven avocado orchards from 2017 to 2019. In each orchard, symptomatic branches were cut from trunks and transported to the laboratory. Small wood pieces were removed from the margins between the healthy and necrotic tissues. The samples were disinfected by immersion in 0.5% NaClO for 30 s, rinsed with sterile water, and then placed in Petri dishes containing 2% water agar. The plates were incubated at 25℃ for 1 to 4 d. Single hyphal tips were transferred to potato dextrose agar (PDA, Difco Inc., Detroit, MI, USA) to obtain a pure culture. All the isolates were stored in 1 mL of sterile water at two different temperatures, 10℃ and 25℃ .

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Mycelia from each isolate were collected from colonies cultivated on PDA and DNA was extracted using QuickExtract™ Plant

DNA Extraction Solution (Epicenter, Madison, WI, USA). The internal transcribed spacer 1 (ITS1)-5.8S-ITS2 region of ribosomal DNA (referred to as the ITS) and partial sequence of the translation elongation factor 1-α (TEF1-α) were amplified using primers ITS4 and ITS5 (White *et al.* 1990), and EF1F and EF2R (Jacobs *et al.* 2004), respectively. For isolate AZP69, sequence of TEF1-α was amplified using primers EF688F and EF1251R (Alves *et al.* 2008). PCR was performed in a 25-μL reaction mixture containing 5 μL of Fast-Run™ Taq Master Mix 5× (Protech Technology Enterprise Co., Ltd., Taipei, Taiwan), 18 μL of ddH_2O, 0.5 μL of each primer (10 pmole μL^{-1}), and 1 μL of DNA template (50 ng μL^{-1}). The PCR conditions for the primers ITS4 and ITS5 were 4 min of initial denaturation at 94℃, followed by 35 cycles of 30 s of denaturation at 94℃, 30 s of annealing at 52℃, and 30 s of extension at 72℃, and a final extension of 4 min at 72℃. For the primers EF1F, EF2R, EF688F and EF1251R, the PCR conditions were 4 min of initial denaturation at 94℃, followed by 35 cycles of 30 s of denaturation at 94℃, 30 s of annealing at 55℃, and 45 s of extension at 72℃, and a final extension of 5 min at 72℃. The PCR product was purified and sequenced by Tri-I Biotech, Inc. (Taipei, Taiwan).

Phylogenetic analyses

Bayesian inference was used to construct phylogenetic trees. The ITS and TEF1-α nuclear gene regions were used in the analysis. Sequences of 56 *Lasiodiplodia* isolates from the GenBank database were included in the tree (Table 1). Multiple sequence alignment of each gene was conducted using ClustalX v. 2.1 (Larkin *et al.* 2007), and these alignments were concatenated using SequenceMatrix v. 2.1.10 (Vaidya *et al.* 2011). Modeltest (Darriba *et al.* 2012) was used to choose the best-fit DNA substitution model under the Bayesian information criterion (BIC). The DNA substitution model used for ITS and TEF1-α were K80 + I and GTR + G, respectively. Phylogenetic tree construction was conducted with MrBayes v. 3.2.6 (Ronquist *et al.* 2012). The analysis was run twice for 1×10^7 generations, and samples were taken from the posterior every 1,000 generations. The first 25% of generations were discarded as burn-in. The tree is rooted with *Barriopsis fusca* CBS174.26. The tree and matrices were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S27172).

Morphological characterization

Colony morphology was characterized by growing isolates on PDA for 3 d at 25℃ in darkness. Production of pycnidia and conidia was induced by placing mycelial plugs on 2% (w/v) water agar (Merck, Darmstadt, Germany) supplemented with autoclaved horsetail stem (*Casuarina equisetifolia* L.) (Ni *et al.* 2011; Kee *et al.* 2017) and incubating for over 21 d at 25℃ under black light (TL-D 18W BLB, Philips, Amsterdam, Netherlands). Morphological characteristics (e.g., color, shape, and septation of conidia and paraphyses) were observed with a Nikon 80i microscope (Tokyo, Japan), and pictures were captured with a Progres Gryphax camera (Jenoptik, Jena, Germany). The length and width of 50 conidia were measured.

Effect of temperature on mycelial growth

Mycelial discs (5 mm in diameter) of thirteen isolates obtained from necrotic avocado wood tissues (Table 2) were transferred to PDA in Petri dishes (9 cm in diameter) and incubated at 10, 15, 20, 25, 30 and 35℃ in darkness. There were six replicates for each temperature. The colony diameter was measured at 24 h.

Pathogenicity test on stems

One-year-old grafted 'Hall' avocado plants were kept in a greenhouse (ambient temperature 25–45℃). Healthy stems about 1 cm diameter were selected for inoculation. The surface of the stem was disinfected with 75% ethanol. A piece of bark (5 mm in diameter) was removed from the stem with a sterilized cork borer. *L. theobromae* ADN21 and *Lasiodiplodia pseudotheobromae* AZC38 were selected for the pathogenicity test as they were isolated from the two

Table 1. GenBank accession numbers for DNA sequences of *Lasiodiplodia* spp. used in the phylogenetic analyses.

		GenBank accession number[z,y]	
Species	Isolate	ITS	TEF1-α
Barriopsis fusca	CBS 174.26	KF766149	KF766395
Lasiodiplodia brasiliense	COAD 1784	KP244693	KP308469
L. brasiliense	COAD 1786	KP244696	KP308471
L. brasiliense	COAD 1787	KP244695	KP308470
L. brasiliense	CDA 431	KP244694	KP308472
L. brasiliense	CMM4015	JX464063	JX464049
Lasiodiplodia crassispora	CBS110492	EF622086	EF622066
L. crassispora	CMW13488	FJ888465	FJ888452
Lasiodiplodia egyptiacae	BOT29	JN814401	JN814428
L. egyptiacae	CBS130992	JN814397	JN814424
L. egyptiacae	COAD 1791	KP244687	KP308462
Lasiodiplodia euphorbiicola	CMM3652	KF234554	KF226715
L. euphorbiicola	CMM3609	KF234543	KF226689
Lasiodiplodia exigua	CBS137785	KJ638317	KJ638336
L. exigua	BL185	KJ638319	KJ638338
Lasiodiplodia gilanensis	IRAN1523C	GU945351	GU945342
L. gilanensis	IRAN1501C	GU945352	GU945341
Lasiodiplodia gonubiensis	CBS115812	DQ458892	DQ458877
Lasiodiplodia hormozganensis	IRAN1500C	GU945355	GU945343
L. hormozganensis	IRAN1498C	GU945356	GU945344
Lasiodiplodia iraniensis	IRAN 921C	GU945346	GU945334
L. iraniensis	IRAN 1519C	GU945350	GU945338
Lasiodiplodia jatrophicola	CMM3610	KF234544	KF226690
Lasiodiplodia macrospora	CMM3833	KF234557	KF226718
L. mahajangana	CMW27801	FJ900595	FJ900641
L. mahajangana	CMW27820	FJ900597	FJ900643
L. missouriana	UCD2193M	HQ288225	HQ288267
L. missouriana	UCD2199MO	HQ288226	HQ288268
Lasiodiplodia parva	CBS456.78	EF622083	EF622063
L. parva	CBS494.78	EF622084	EF622064
L. parva	CBS495.78	EF622085	EF622065
Lasiodiplodia plurivora	STEU-5803	EF445362	EF445395
Lasiodiplodia pseudotheobromae	COAD 1785	KP244690	KP308464
L. pseudotheobromae	CBS116459	EF622077	EF622057
L. pseudotheobromae	CBS116460	EF622078	EF622058
L. pseudotheobromae	CMM3887	KF234559	KF226722
L. pseudotheobromae	CBS 447.62	EF622081	EF622060

Table 1. GenBank accession numbers for DNA sequences of *Lasiodiplodia* spp. used in the phylogenetic analyses. (continued)

		GenBank accession number[z,y]	
Species	Isolate	ITS	TEF1-α
L. pseudotheobromae	**AZC38**	**MN911394**	**MN921243**
L. pseudotheobromae	**ADN23**	**MN911393**	**MN921242**
L. pseudotheobromae	**AZC12**	**MN911392**	**MN921241**
L. pseudotheobromae	**AFL8**	**MT093794**	**MT086519**
Lasiodiplodia pyriformis	CBS121770	EU101307	EU101352
L. pyriformis	CBS121771	EU101308	EU101353
Lasiodiplodia subglobosa	CMM3872	KF234558	KF226721
L. subglobosa	CMM4046	KF234560	KF226723
Lasiodiplodia theobromae	CBS111530	EF622074	EF622054
L. theobromae	CBS 164.96	AY640255	AY640258
L. theobromae	CBS 124.13	DQ458890	DQ458875
L. theobromae	CBS 339.90	EF622072	EF622052
L. theobromae	CMW9074	AY236952	AY236901
L. theobromae	COAD 1788	KP244698	KP308476
L. theobromae	COAD 1789	KP244700	KP308474
L. theobromae	CDA 425	KP244697	KP308475
L. theobromae	CDA 444	KP244699	KP308477
L. theobromae	CDA 450	KP244688	KP308478
L. theobromae	CDA 455	KP244689	KP308463
L. theobromae	CDA 467	KP244702	KP308473
L. theobromae	**ADN21**	**MN911388**	**MN921237**
L. theobromae	**ACY1**	**MN911389**	**MN921238**
L. theobromae	**ACY4**	**MN911390**	**MN921239**
L. theobromae	**AZC42**	**MN911391**	**MN921240**
L. theobromae	**AZP58**	**MT093789**	**MT086514**
L. theobromae	**AZP69**	**MT093790**	**MT086515**
L. theobromae	**AFL1**	**MT093791**	**MT086516**
L. theobromae	**ASS25**	**MT093792**	**MT086517**
L. theobromae	**ASS28**	**MT093793**	**MT086518**
Lasiodiplodia viticola	UCD2604MO	HQ288228	HQ288270
L. viticola	UCD2553AR	HQ288227	HQ288269
Lasiodiplodia venezuelensis	CBS118739	DQ103547	DQ103568
L. venezuelensis	CMW13512	DQ103549	DQ103570

[z] ITS: internal transcribed spacer regions 1 and 2, including the 5.8S ribosomal ribonucleic acid (RNA) gene; and TEF1-α: translation elongation factor 1-α.

[y] Isolates in boldface were obtained in this study.

Table 2. Conidial measurements of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* obtained from avocado in Taiwan and those obtained in previous studies

Species	Isolate	Location	Conidial size (μm)			Source
			Length[z]	Width[y]	L/W[x]	
L. theobromae	ADN21	Danei, Tainan	20.5-24.6-28.3	12.5-14.0-15.5	1.8	This study
	ACY1	Chiayi	19.4-24.0-28.8	10.7-13.9-17.9	1.8	This study
	ACY4	Chiayi	19.1-22.2-24.5	12.0-13.4-15.5	1.7	This study
	AZC42	Zhuqi, Chiayi	20.2-22.6-25.3	11.9-13.5-14.5	1.7	This study
	AZP58	Zhongpu, Chiayi	19.0-23.6-27.2	11.1-13.4-15.5	1.8	This study
	AZP69	Zhongpu, Chiayi	22.1-24.7-27.3	10.8-13.3-14.9	1.9	This study
	AFL1	Fanlu, Chiayi	21.2-24.4-28.4	12.3-13.8-15.7	1.8	This study
	ASS25	Shanshang, Tainan	19.1-21.6-24.8	11.1-12.3-14.1	1.8	This study
	ASS28	Shanshang, Tainan	21.3-24.2-27.9	11.5-13.2-15.3	1.8	This study
	-	-	21.0-26.2-31.0	13.0-14.2-15.0	1.9	Alves *et al.* (2008)
			22.4-24.2	12.9-14.3	1.8	Abdollahzadeh *et al.* (2010)
			24.49-27.49	13.30-14.79	1.8	Marques *et al.* (2013)
	CBS 164.96	-	19.0-26.2-32.5	12.0-14.2-18.5	1.9	Phillips *et al.* (2013)
			20.7-22.7	11.7-14.1	1.8	Netto *et al.* (2014)
	-	-	24.5-28.2	13.3-15.1	1.8	Correia *et al.* (2016)
	-	-	18.5-30.5	12-18	-	Rosado *et al.* (2016)
	PALUC449F	-	16.7-20.9-26.2	11.1-12.7-14.5	1.7	Valencia *et al.* (2019)
L. pseudotheobromae	AZC38	Zhuqi, Chiayi	21.1-25.8-32.7	12.4-14.4-18.1	1.8	This study
	ADN23	Danei, Tainan	22.4-24.9-30.3	10.1-12.9-14.4	2.0	This study
	AZC12	Zhuqi, Chiayi	19.3-22.5-25.6	11.2-13.6-15.5	1.7	This study
	AFL8	Fanlu, Chiayi	22.9-26.4-30.0	11.9-16.3-19.3	1.6	This study
			21.7-26.3	13.4-14.8	1.7	Abdollahzadeh *et al.* (2010)
			25.07-28.23	13.4-15.6	1.8	Marques *et al.* (2013)
			21.2-25.8	12.5-13.9	1.8	Netto *et al.* (2014)
	-	-	23.5-28.0-32.0	14-16-18	1.7	Alves *et al.* (2008)
	-	-	25.3-29.6	14.7-16.8	1.8	Correia et al. (2016)
	-	-	25-32	14-18	-	Rosado et al. (2016)

[z] Minimum length-average length-maximum length (or minimum length-maximum length when the average was not available).
[y] Minimum width-average width-maximum width (or minimum width-maximum width when the average was not available).
[x] L/W = average length/average width.

major avocado production areas in Taiwan. A 2-day-old mycelial plug (5 mm in diameter) was placed in the wound with the mycelial side facing the wound. Control plants were treated with sterile PDA discs (5 mm in diameter). The wound was sealed with parafilm to prevent dehydration, which was unwrapped at 2 wk after inoculation. The experimental design was completely randomized with 10 replicates for each treatment. The lengths of the external necrotic lesions were measured weekly. After 4 wk, the stems were cut and the lengths of the lesions that developed inside the stem were measured. Pathogens were re-isolated from lesions and re-identified by analysis of colony morphology and ITS sequences to fulfill Koch's postulates. The experiment was repeated twice.

Pathogenicity test on fruits

Two local cultivars, 'Changan' and 'Zhongpu Green Skin', were chosen for pathogenicity test because their fruits remain green-skinned after ripening, making lesion observation more convenient. The fruits were harvested at maturity and surface disinfected with 75% ethanol. Two-day-old mycelial plugs (5 mm in diameter) of *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 were placed on the fruits with the mycelial side facing the fruits. Control fruits were treated with sterile PDA discs (5 mm in diameter). The fruits were either unwounded or wounded with a flamed-sterilized needle (5 mm in depth). There were 5 replicates for each treatment. The fruits were kept at 25℃ in 100% relative humidity for the first 2 d after inoculation, and then the relative humidity were reduced to 65% for the rest of the trial. The diameters of the necrotic lesions on fruits were measured at 3 and 5 d after inoculation, and the data were subjected to one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test using SAS Enterprise Guide version 7.15. Pathogens were re-isolated from lesions and re-identified by analysis of colony morphology and TEF1-α sequences.

RESULTS

Field symptoms and fungi isolation

A total of 13 isolates were recovered from symptomatic avocado branches from seven different orchards (Table 2). The symptoms included dieback of affected branches (Fig. 1A) and internal browning visible in the cross section of a wilted branch (Fig. 1B). Darkening of bark with extrusion of reddish sap also occurred, and the wood underneath the bark turned reddish brown (Figs. 1C–1D). In addition, there was evidence

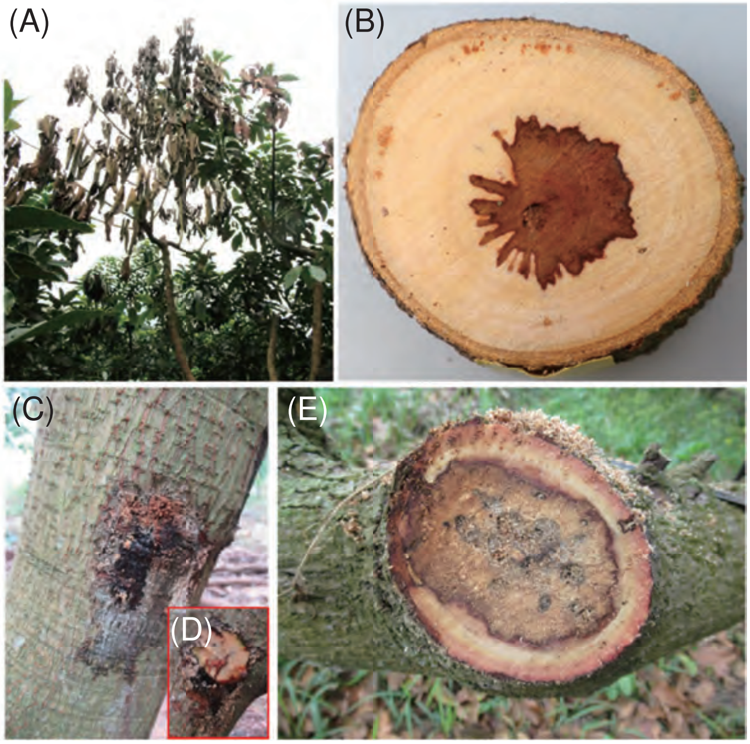

Fig. 1. Symptoms of avocado branch canker in the field. (A) Dieback of an affected branch; (B) internal browning in a wilted branch; (C) discolored bark with reddish sap; (D) reddish brown wood underneath the bark; and (E) browning and decay visible in the cross section of an old pruning wound.

that infection could also start from a mechanical injury, such as a pruning cut. For example, when pruning was not done properly, i.e., cutting too far from the branch collar and leaving a section of dead limb on the tree, it appears that the pruning wounds did not heal properly and infection occurred, making the inner tissues turn brown and decay (Fig. 1E).

Phylogenetic analysis

DNA sequences of the two gene regions were obtained from 70 isolates included in this study and concatenated to form a supermatrix of 1,347 bps. The result revealed the thirteen avocado isolates in this study belonged to *L. theobromae* or *L. pseudotheobromae* (Fig. 2). Four isolates, AND23, AFL8, AZC12 and AZC38, were under the clade *L. pseudotheobromae* with a high Bayesian posterior probability (BPP) value of 100%. Nine *L. theobromae* isolates (ACY1, ACY4, AFL1, ADN21, ASS25, ASS28, AZC42, AZP58 and AZP69) were under *L. theobromae* clade.

Morphological characterization

The conidial size of each isolate is shown in Table 2. *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 were selected for further morphological characterization, as they were also tested for pathogenicity. Colonies of *L. theobromae* ADN21 grown on PDA were greyish white on the upper surface with fluffy aerial mycelia, and the lower surface was greyish white initially but became dark green after 3 d (Figs. 3A–3B). Paraphyses were hyaline, cylindrical, and aseptate. Conidia were subovoid to ellipsoid. Immature conidia were hyaline and aseptate, and mature conidia were dark brown and 1-septate, measuring 24.6 μm × 14.0 μm on average (n = 50) (Table 2, Figs. 3C–3E). Colonies of *L. pseudotheobromae* AZC38 grown on PDA were also grey-white on the upper surface with fluffy aerial mycelia, but the lower surface remained greyish white after 3 d (Figs. 4A–4B). Paraphyses were hyaline, cylindrical, and aseptate. Conidia were subovoid to ellipsoid. Immature conidia were hyaline and aseptate, and mature conidia were dark brown and 1-septate, measuring 25.8 μm × 14.4 μm on average (n = 50) (Table 2, Figs. 4C–4E).

Effect of temperature on mycelial growth

All 13 isolates of *L. theobromae* and *L. pseudotheobromae* could grow in the range of 10–35℃. *L. theobromae* grew slightly faster than *L. pseudotheobromae* on average, but not all isolates of *L. theobromae* grew faster than *L. pseudotheobromae* isolates (Fig. 5). Optimal mycelial growth for both species was observed at 30℃; the average colony diameters of *L. theobromae* and *L. pseudotheobromae* at this temperature were 58.9 mm and 53.8 mm, respectively.

Pathogenicity test on stems

Both *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 were pathogenic to 'Hall' avocado. After inoculation with *L. theobromae* ADN21 or *L. pseudotheobromae* AZC38, excretion of a white powder and an external black lesion were observed at the inoculation site of every stem, while the inoculation site in the control treatment healed by the end of trial (Figs. 6A–6C). The external black lesions at the *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 inoculation sites expanded by 0.79 cm and 1.10 cm, respectively, from week 2 to week 4 (Table 3). Underneath the lesions, the tissues turned brown along the vascular bundle, and the internal lesion lengths were longer the external lesions (Figs. 6E–6F). The average internal lesion lengths were 9.98 cm and 8.79 cm for *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38, respectively, at 4 wk after inoculation (Table 3). Both isolates were successfully re-isolated from necrotic tissues and re-identified based on cultural features and ITS sequences, while no *Lasiodiplodia* spp. were isolated from the control-treated tissues.

Pathogenicity test on fruits

Both *L. theobromae* ADN21 and *L. pseudotheobromae* AZC38 caused black lesions on avocado fruits. The external lesions were visible at 2 d after inoculation, and continued becoming larger. Underneath the lesions, the pulp turned soft, water-soaked, and black. In wounded in-

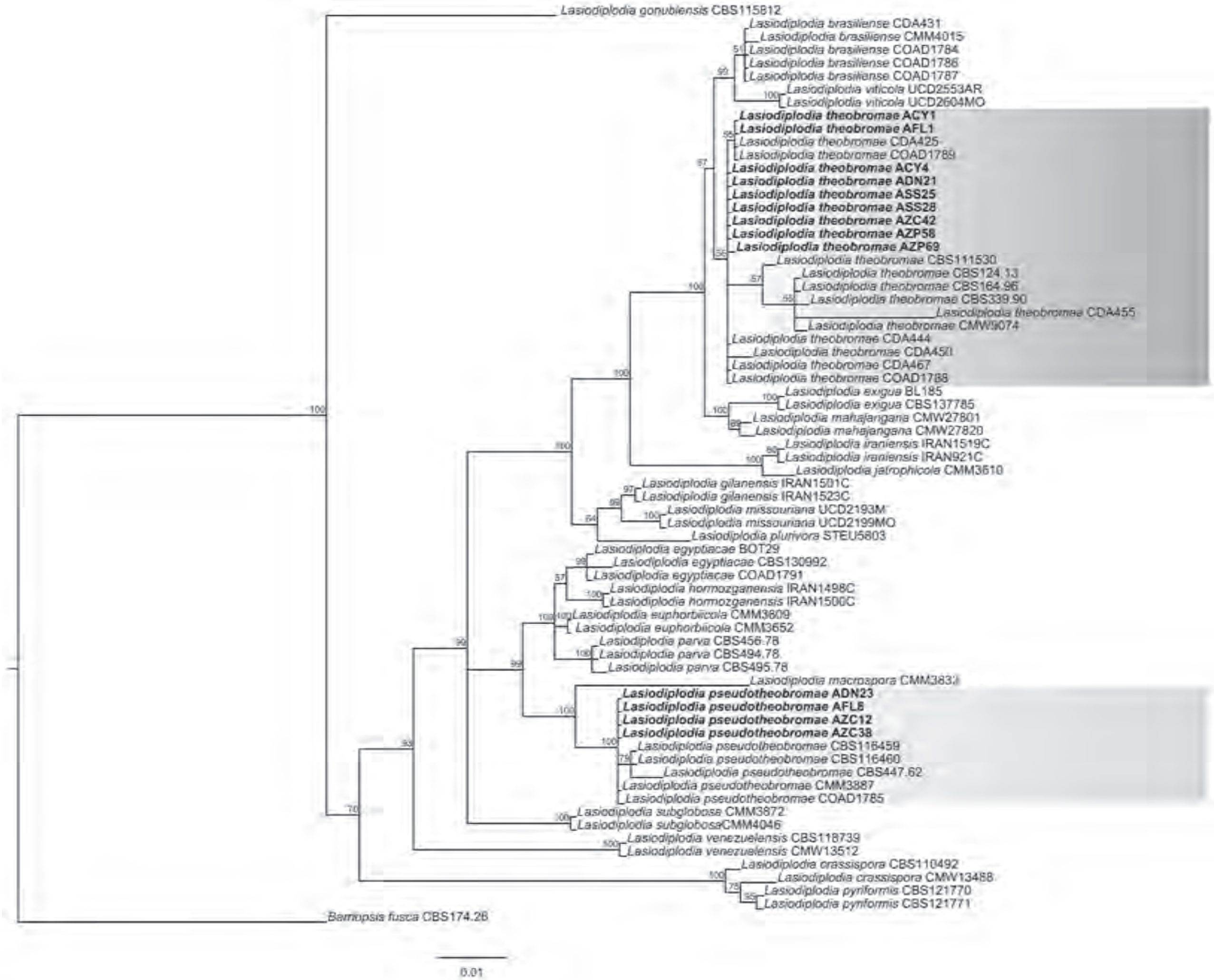

Fig. 2. Bayesian phylogenetic trees of *Lasiodiplodia* isolates from avocado in Taiwan and from GenBank database. The phylogenetic tree was built using concatenated sequences of partial internal transcribed spacer and translational elongation factor 1-α. The Bayesian posterior probabilities are indicated next to the nodes. The tree is rooted by *Barriopsis fusca* CBS174.26. Isolates from this study are emphasized in bold.

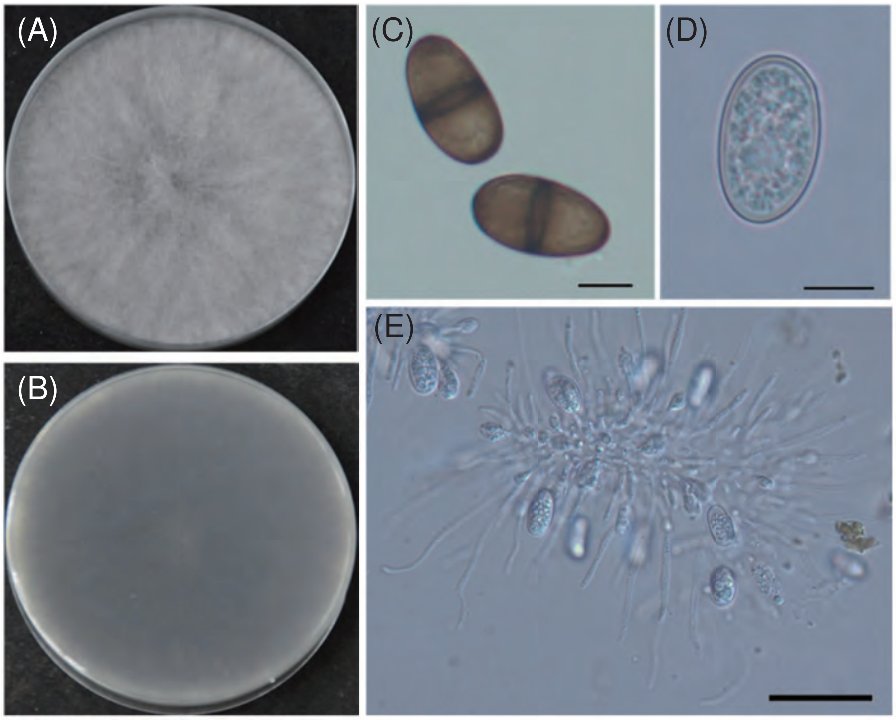

Fig. 3. Morphological characteristics of *Lasiodiplodia theobromae* ADN21. (A–B) Colony morphology on potato dextrose agar after 3 days at 25℃. (A) Top view; (B) bottom view; (C) mature conidia; (D) immature conidia; and (E) conidiogenous layer with conidiogenous cells and paraphyses. Scale bars: (C) and (D) = 10 μm; (E) = 50 μm.

oculation, both isolates caused lesions on both 'Changan' and 'Zhongpu Green Skin' fruits. At 3 d post inoculation, the average lesion diameter caused by *L. pseudotheobromae* AZC38 on 'Changan' fruits was 3.54 cm, which was significantly larger than the lesion caused by *L. theobromae* ADN21 (1.47 cm), while there was no significant difference between them on 'Zhongpu Green Skin' fruits. At 5 d post inoculation, both species caused lesions greater than 6 cm in diameter on both 'Changan' and 'Zhongpu Green Skin' fruits (Table 4). In unwounded inoculation, *L. pseudotheobromae* AZC38 caused lesions on both cultivars, while *L. theobromae* ADN21 only caused lesions on two 'Zhongpu Green Skin' fruits. Both isolates were successfully re-isolated from necrotic pulps and re-identified based on cultural features and TEF1-α sequences. All control fruits remained symptomless at treated sites, and no fungi were re-isolated.

DISCUSSION

Avocado branch canker is widely distributed in many avocado production areas in Taiwan. In this study, *L. theobromae* and *L. pseudotheobromae* were identified from symptomatic avocado branches. To our knowledge, this is the first report characterizing avocado branch canker in Taiwan.

The morphological characteristics of *L. theobromae* and *L. pseudotheobromae* isolates examined in this study generally corresponded to those reported in published studies (Alves *et al.* 2008; Phillips *et al.* 2013; Correia *et al.* 2016; Rosado *et al.* 2016; Valencia *et al.* 2019), although the conidial sizes were slightly smaller. The possible reasons might include different culture methods and differences between isolates. The morphology of *L. pseudotheobromae* is similar to that of *L. theobromae*. In fact, *L. pseudotheobromae* was previously considered

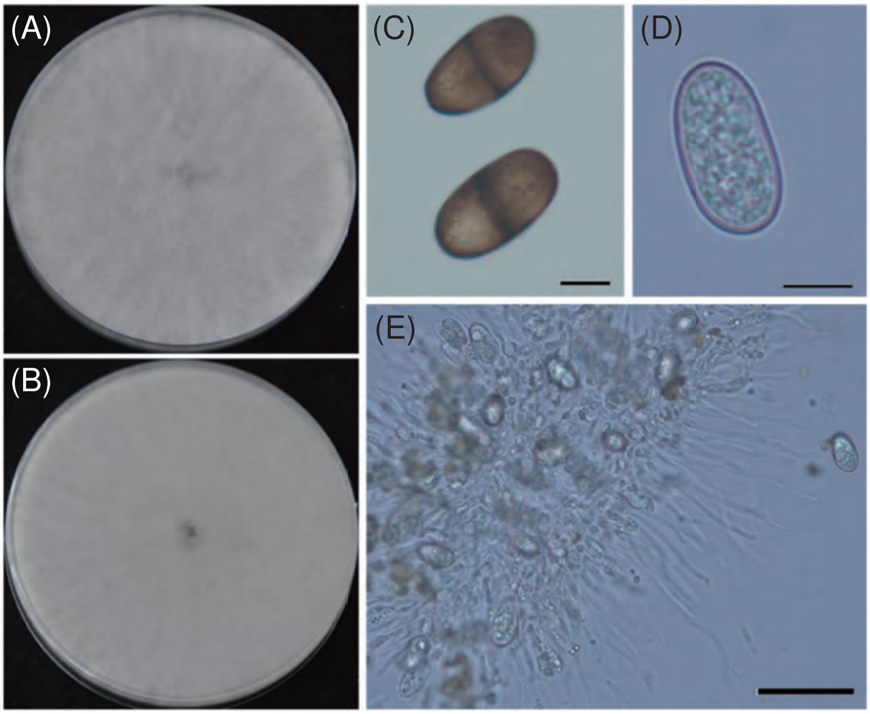

Fig. 4. Morphological characteristics of *Lasiodiplodia pseudotheobromae* AZC38. (A–B) Colony morphology on potato dextrose agar after 3 days at 25℃ (A, Top view; B, Bottom view); (C) mature conidia; (D) immature conidia; and (E) conidiogenous layer with conidiogenous cells and paraphyses. Scale bars: (C–D) = 10 μm; (E) = 50 μm.

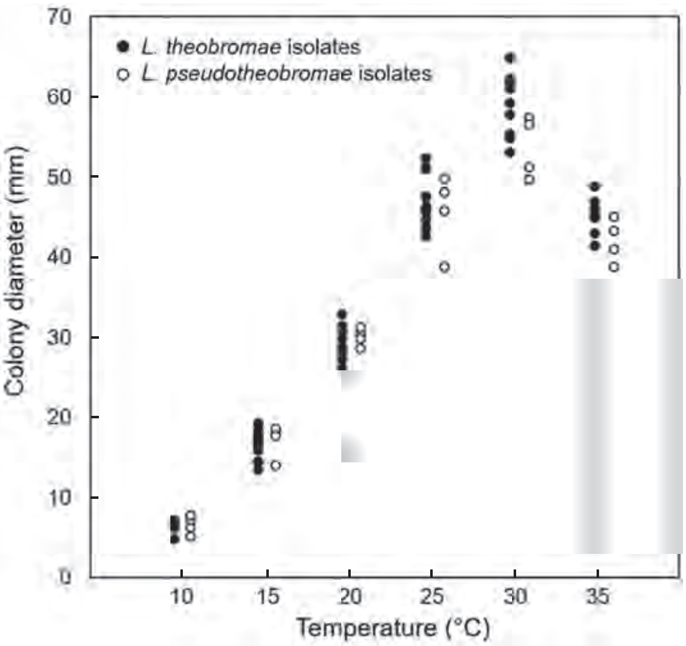

Fig. 5. Effect of temperature on mycelial growth of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* isolated in this study. Colony diameter (mm) was measured at 24 h after inoculation on potato dextrose agar. Each dot represents the average colony diameter for each isolate from six replicates.

to be *L. theobromae* until Alves *et al.* (2008) combined morphological and phylogenetic analyses to distinguish these species. According to Alves *et al.* (2008), *L. pseudotheobromae* differs from *L. theobromae* in its conidia, which are larger and more ellipsoid and do not taper as strongly toward the truncate base; in addition, *L. pseudotheobromae* can grow at 10℃. However, according to our study and previous studies, conidial shape ranges from subovoid to ellipsoid for all isolates, and conidial size also varies between different isolates. As for the differences in growth at low temperature, all isolates of both species in the present study could grow at 10℃, which is consistent with the findings of Abdollahzadeh *et al.* (2010), Marques *et al.* (2013) and Netto *et al.* (2014).

Fig. 6. (A–C) External lesions and (D–F) internal discoloration that developed 4 wk after artificial inoculation on 'Hall' avocado stems. (A, D) Control; (B, E) *Lasiodiplodia theobromae* ADN21; and (C, F) *Lasiodiplodia pseudotheobromae* AZC38.

Because morphological and culture characteristics might not be sufficient to distinguish closely related species of *Lasiodiplodia*, it is necessary to analyze sequences from multiple loci to identify *Lasiodiplodia* spp. Thirteen isolates obtained from different avocado growing areas in Taiwan were included in the phylogenetic analyses. All of them were identified as either *L. theobromae* or *L. pseudotheobromae* based on ITS and TEF-1α sequences.

The pathogenicity test on stems in this study showed that *L. theobromae* and *L. pseudotheobromae* are pathogenic to the avocado 'Hall' cultivar. Under the conditions used in the test, symptoms developed rapidly and lesions were visible after 2 wk of inoculation. Avocado branch canker has been reported to be associated with several fungal species in different countries, most of which are members of the Botryosphaeriaceae family, including *L. pseudotheobromae*, *N. austral*, *N. luteum*, *N. parvum*, *N. nonquaesitum*, *F. aesculi*, *D. iberica*, *Dip. mutila*, *Dip. pseudoseriata*, and *Dip. seriata* (McDonald & Eskalen 2011; Eskalen *et al.* 2013; Trakunyingcharoen *et al.* 2015; Guarnaccia *et al.* 2016). Interestingly, *L. theobromae* was not reported as a major pathogen in most previous studies investigating pathogens of avocado branch canker or

Table 3. Pathogenicity test of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* on 'Hall' avocado stem.

	Lesion length (M ± SD, cm)[z]				
Isolate	2 wk[y]	3 wk[y]	4 wk[y]	4 wk[x]	Incidence[w]
L. theobromae ADN21	3.60 ± 1.23	4.19 ± 1.71	4.39 ± 1.77	9.98 ± 4.03	10/10
L. pseudotheobromae AZC38	5.29 ± 3.03	6.24 ± 4.02	6.39 ± 4.00	8.79 ± 3.79	10/10
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.38	0/10

[z] Mean (M) and standard deviation (SD) are derived from 10 replicates.
[y] External lesions at weeks 2, 3 and 4.
[x] Internal lesions at 4 wk.
[w] Incidence = number of stems with external lesions/number of treated stems.

Table 4. Pathogenicity test of *Lasiodiplodia theobromae* ADN21 and *Lasiodiplodia pseudotheobromae* AZC38 on avocado fruits.

		Lesion diameter (M ± SD, cm)[z]					
		'Changan'			'Zhongpu Green Skin'		
Isolate	Wound[y]	3 d	5 d	Incidence[x]	3 d	5 d	Incidence[x]
L. theobromae ADN21	+	1.47 ± 1.08 b	6.24 ± 1.95 a	5/5	2.97 ± 0.95 a	7.45 ± 1.76 a	5/5
	−	0.00 ± 0.00 c	0.00 ± 0.00 c	0/5	0.44 ± 0.64 b	1.35 ± 2.09 b	2/5
L. pseudotheobromae AZC38	+	3.54 ± 0.63 a	7.83 ± 0.99 a	5/5	3.20 ± 0.44 a	8.00± 1.52 a	5/5
	−	1.42 ± 1.04 b	4.09 ± 2.37 b	4/5	2.51 ± 0.82 a	6.66 ±1.15 a	5/5
Control	+	0.00 ± 0.00 c	0.00 ± 0.00 c	0/5	0.00 ± 0.00 b	0.00 ± 0.00 b	0/5
	−	0.00 ± 0.00 c	0.00 ± 0.00 c	0/5	0.00 ± 0.00 b	0.00 ± 0.00 b	0/5

[z] Mean (M) and standard deviation (SD) are derived from 5 replicates. Means in the same column followed by the same letter are not significantly different according to Fisher's least significant difference (LSD) test ($P = 0.05$).

[y] "+" means wounded inoculation, while "−" means unwounded inoculation.

[x] Incidence = number of symptomatic fruits/number of treated fruits.

dieback (McDonald & Eskalen 2011; Eskalen *et al.* 2013; Guarnaccia *et al.* 2016), and seemed to be a minor avocado branch canker pathogen (Arjona-Girona *et al.* 2019) or more closely associated with stem-end rot (Valencia *et al.* 2019). *L. pseudotheobromae* has only been demonstrated to cause avocado stem canker in Thailand (Trakunyingcharoen *et al.* 2015).

The tropical climate of Taiwan might be one possible reason for the differences in pathogen species between Taiwan and most other countries. First of all, the avocado cultivars planted in Taiwan are mostly the West Indian race or West Indian × Guatemalan hybrids because they perform better in tropical areas, while major avocado production countries mostly plant 'Hass', a Guatemalan × Mexican hybrid. Similar to Taiwan, *L. pseudotheobromae* was also demonstrated to cause stem canker on avocado in Thailand (Trakunyingcharoen *et al.* 2015), where the most common avocados planted are West Indian × Guatemalan hybrids (Babpraserth & Subhadrabandhu 2000). Whether the virulence and distribution of Botryosphaeriaceae spp. on avocado stems varies among different cultivars requires further studies.

Secondly, the higher temperature in Taiwan might also result in the difference in pathogenic species between Taiwan and other countries. Previous studies indicated that climatic factors might not explain the distribution of Botryosphaeriaceae spp. on avocado because the same species could be detected in avocado orchards located in different climatic zones (McDonald & Eskalen 2011; Valencia *et al.* 2019). However, a phylogeographic study on the distribution of Botryosphaeriaceae in Australia indicated that although some species had a wide distribution across tropical and temperate regions, most *Lasiodiplodia* spp. were more common in tropical regions, while *Diplodia*, *Dothiorella* and most *Neofusicoccum* spp. were more common in temperate regions (Burgess *et al.* 2019). In addition, the optimal growth temperature of *Diplodia* spp. and *Neofusicoccum* spp. was around 25℃ (Valencia *et al.* 2019), while it was found to be around 30℃ for both *L. theobromae* and *L. pseudotheobromae* in this study and previously published studies (Netto *et al.* 2014; Valencia *et al.* 2019).

The annual average temperature in Tainan City, the major avocado production area in Taiwan, was around 25℃ in 2018, with a high temperature of 33℃ and low temperature of 14℃ (Central Weather Bureau 2018). The average temperature is clearly higher than that in temperate avocado production countries. For example, the average temperature of avocado production areas is around 14℃ in Chile (Barros & Sanchez 1992) and 13–18℃ in California (McDonald & Eskalen 2011). Therefore, it is possible that the warmer tropical climate in Taiwan might favor

the growth of *L. theobromae* and *L. pseudotheobromae*, resulting in the difference in pathogenic species compared with other countries. Similarly, *L pseudotheobromae* was also demonstrated to cause stem canker on avocado in Thailand (Trakunyingcharoen *et al.* 2015), which is also a tropical area. Further investigation of the fungal species associated with avocado branch canker in tropical regions would be helpful for understanding the epidemiology of Botryosphaeriaceae species on avocado and developing avocado disease management strategies in tropical regions.

The pathogenicity test on fruits in this study showed that *L. pseudotheobromae* caused lesions on both wounded and unwounded inoculated fruits, while *L. theobromae* only caused severe symptoms on wounded fruits. Though previous studies had demonstrated that both *L. theobromae* and *L. pseudotheobromae* were pathogenic to avocado fruits (Ni *et al.* 2011; Trakunyingcharoen *et al.* 2015; Valencia *et al.* 2019), this study further indicated that *L. pseudotheobromae* could invade avocado fruits without a wound. Therefore, *L. pseudotheobromae* might cause postharvest fruit rot not only at stem-ends, but also at other fruit parts.

As avocado branch canker is a major threat to avocado production worldwide, it is crucial to develop effective control methods. The results of this study suggest that the fungal pathogens associated with avocado branch canker might differ between tropical and temperate regions, indicating that in the future it is important to study the epidemiology of the pathogens and develop effective management strategies.

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Lasiodiplodia theobromae 及 *Lasiodiplodia pseudotheobromae* 在台灣引起之酪梨枝條潰瘍病

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摘要

梁鈺平、吳昭蓉、蔡惠文、倪蕙芳。2021。*Lasiodiplodia theobromae* 及 *Lasiodiplodia pseudotheobromae* 在台灣引起之酪梨枝條潰瘍病。台灣農業研究 70(2):81–97。

酪梨枝條潰瘍病於國外許多酪梨栽培國家均曾報導，主要由多種葡萄座腔菌科 (Botryosphaeriaceae) 之真菌感染引起。本病亦廣泛發生於台灣主要酪梨栽培地區，然病原菌至今尚不明確。本研究自台灣各地共 7 個酪梨園具潰瘍病病徵之枝條組織進行病原菌分離，共收集到 13 個菌株，透過型態鑑定及核糖體內轉錄區間 (internal transcribed spacer; ITS) 和轉譯延長因子 1-α (translation elongation factor 1-α gene; TEF-1α) 之序列進行類緣分析，結果顯示此些菌株均屬於 *Lasiodiplodia theobromae* 或 *Lasiodiplodia pseudotheobromae*。此兩菌株之菌絲最適生長溫度均為約 30℃；對酪梨枝條及果實均具病原性，可於枝條接種處造成白色粉末分泌及內部組織褐化，於果實上亦會造成黑色軟腐病斑。本研究為首篇描述台灣酪梨枝條潰瘍病及其相關病原菌之報告，研究結果顯示台灣之病原菌種類與大部分國外酪梨栽培地區造成酪梨枝條潰瘍病之主要病原菌不同。本研究將可提供未來進行酪梨枝條潰瘍病之流行病學，以及建立其有效之病害管理策略研究時之重要參考資訊。

關鍵詞：酪梨、枝條潰瘍病、*Lasiodiplodia theobromae*、*Lasiodiplodia pseudotheobromae*、台灣。

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十字花科蔬菜重要病毒發生調查與影響蚜蟲傳播效率之條件探討

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摘要

曹麗玉。2021。十字花科蔬菜重要病毒發生調查與影響蚜蟲傳播效率之條件探討。台灣農業研究 70(2):98–107。

台灣田間十字花科蔬菜疑似受病毒感染之病徵，主要包括葉片嵌紋、斑駁、黄化、葉脈壞疽、葉脈腫脹、植株矮化等。利用甜菜西方黄化病毒 (*Beet western yellows virus*; BWYV)、花椰菜嵌紋病毒 (*Cauliflower mosaic virus*; CaMV)、胡瓜嵌紋病毒 (*Cucumber mosaic virus*; CMV)、蘿蔔嵌紋病毒 (*Radish mosaic virus*; RaMV)、蕪菁嵌紋病毒 (*Turnip mosaic virus*; TuMV)、蕪菁輪斑病毒 (*Turnip ring spot virus*; TuRSV) 6 種病毒血清搭配間接酵素連結免疫分析法 (indirect enzyme-linked immunosorbent assay; indirect ELISA) 調查不同病毒於簡易設施內十字花科蔬菜的自然感染情形，病毒檢出率高低依序為蘿蔔、芥菜類及白菜類。其中，病毒種類以 TuMV 檢出率最高，BWYV 次之；複合感染情形以 BWYV 與 TuMV 居多。以上述 6 種血清及間接 ELISA 分析法進行檢測結球白菜、不結球白菜、甘藍、油菜、芥菜、芥藍、花椰菜、青花菜、蘿蔔共 27 品種 40 批號之十字花科蔬菜種子研磨液，未檢測出病毒。以人工接種方式接種 TuMV 單斑分離株於小白菜或葉用蘿蔔，作為偽菜蚜 (*Lipaphis erysimi* Kaltenbach) 的獲毒來源，用以研究媒介昆蟲的傳播特性。結果發現，以 1 隻帶有 TuMV 蚜蟲取食健康葉用蘿蔔 10 d 後即可出現病徵，同時接種 5 隻帶 TuMV 蚜蟲，傳毒率可達 100%；蚜蟲獲毒溫度及接種溫度為 25℃時，傳毒率可達 100%；蚜蟲獲毒取食時間為 10 min，傳毒率可達 100%；若蚜蟲接種取食時間若為 60 min，傳毒率可達 100%。以人工接種方式接種 BWYV 分離病毒株作為偽菜蚜的獲毒來源，5 隻傳毒率則可達 100%；當蚜蟲獲毒溫度為 20℃或接種溫度為 25℃時，傳毒率可達 100%；蚜蟲取食帶毒植株 1 h 即可獲毒，若取食時間達 48 h，傳毒率可達 100%。利用白菜離葉測試市面上 8 種常用殺蟲藥劑對偽菜蚜的防治效果，包括 24.7% 賽速洛寧膠囊水懸混劑 (61.75 mg a.i. L^{-1})、16% 可尼丁水溶性粒劑 (40.00 mg a.i. L^{-1})、25% 賽速安水溶性粒劑 (50.00 mg a.i. L^{-1})、20% 達特南水溶性粒劑 (10.00 mg a.i. L^{-1})、1% 密滅汀乳劑 (6.67 mg a.i. L^{-1})、20% 亞滅培水溶性粉劑 (33.33 mg a.i. L^{-1})、18.2% 益達胺水懸劑 (22.75 mg a.i. L^{-1})、50% 培丹水溶性粉劑 (500.00 mg a.i. L^{-1}) 等，噴灑 48 h 後，蚜蟲防治率均可達 87% 以上。以清潔劑及油類等非農藥防治資材評估對於蚜蟲的防治效果，以 35% 無患子濃縮液稀釋 50× 及 99% 礦物油稀釋 300× 的蚜蟲防治率較佳。

關鍵詞：十字花科蔬菜、蕪菁嵌紋病毒、甜菜西方黃化病毒、蚜蟲傳播。

前言

十字花科植 (作) 物 (family Cruciferae or Brassicaceae) 種類繁多，地理分布極廣，除南極洲外廣泛分布在世界各地。科內作物包含食用作物、香料作物 (如：辣根屬的辣根 *Aremoracia rusticana*、山葵屬的山葵 *Wasabia japonica*)、油料作物 (蕓苔屬的油菜 *Brassica napus*) 及試驗模式作物 (阿拉伯芥屬的阿拉伯芥 *Arabidopsis thaliana*) 等。十字花科作物以蕓苔屬 (Genus *Brassica*) 最具經濟重要性，包括結球白菜 (*Brassica rapa* subsp. *pekinensis*)、不結球白菜 (*Brassica*

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rapa subsp. *chinensis*)、芥菜 (*Brassica juncea*)、大頭芥 (*Brassica juncea* var. *megarrhiza*)、榨菜 (*Brassica juncea* var. *tumida*)、芥藍 (*Brassica oleracea* var. *alboglaba*)、花椰菜 (*Brassica oleracea* var. *botrytis*)、甘藍 (*Brassica oleracea* var. *capitata*)、球莖甘藍 (*Brassica oleracea* var. *caulorapa*)、青花菜 (*Brassica oleracea* var. *italica*)、蕪菁 (*Brassica rapa* subsp. *rapa*)，以及蘿蔔屬的蘿蔔 (*Raphanus sativus*) (Anjum *et al.* 2012)。依據 2019 年台灣農業統計年報資料 (https://agrstat.coa.gov.tw/sdweb/public/book/Book.aspx) 記載：台灣地區全年作不結球白菜種植面積約 5,466 ha，年產量約 106,296 Mt，其中以中部的雲林縣、北部的桃園市及南部的高雄市為主要栽培區，分別占全國栽培面積的 55.9、23.7 及 5.6%。結球白菜全年作種植面積約 1,808 ha，年產量約 73,219 Mt，其中以中部的雲林縣、彰化縣、北部的宜蘭縣及南部的高雄市為主要栽培區，分別占全國栽培面積的 48.2、21.8、4.0 及 3.3%。甘藍全年作種植面積約 8,392 ha，年產量約 427,738 Mt，其中以中部的雲林縣、南投縣及北部的宜蘭縣為主要栽培區，分別占全國栽培面積的 22.5、16.8 及 15.2%。

依據早期國外文獻記載，為害十字花科作物之病毒共有 26 種 (Lister 1958; Brunt *et al.* 1996)，其中有部分病毒會造成重大危害及經濟損失 (Duffus 1960; Gladders 1987; Hill *et al.* 1989; Stobbs & Shattuck 1989)。台灣地區有關十字花科作物之病毒病研究最初開始於 Kou (1961) 對於蘿蔔嵌紋病病因之探討，後續之文獻記錄則有胡瓜嵌紋病毒 *Cucumber mosaic virus* (CMV) (Chen 1975)、蕪菁嵌紋病毒 *Turnip mosaic virus* (TuMV) (Lin & Lian 1983)、花椰菜嵌紋病毒 *Cauliflower mosaic virus* (CaMV) (Chen *et al.* 2000)、甜菜西方黃化病毒 *Beet western yellows virus* (BWYV) (Chen 2003)、蘿蔔嵌紋病毒 *Radish mosaic virus* (RaMV) (Wang *et al.* 2014) 及蕪菁輪紋病毒 *Turnip ring spot virus* (TuRSV) (Chen *et al.* 2011)。根據報告記載，為害蘿蔔之病毒有 TuMV、CMV、RaMV 等 3 種，其中以 TuMV 為害最普遍，且最具有經濟重要性 (Green & Deng 1985)。又根據 Chen *et al.* (2000) 田間病株以雙抗體酵素免疫分析法 (double antibody sandwich enzyme-linked immunosorbent assay; DAS-ELISA) 檢測，可檢出 TuMV、BWYV、CaMV 等 3 種，且病毒檢出率高低之作物種類依序為蘿蔔、芥菜類及白菜類，皆可高達 60% 以上。由於台灣以往對十字花科作物病毒之研究並未全面調查，而近十餘年來台灣農業栽培生態已發生改變，作物品種朝向多樣化選擇，本研究乃針對台灣南部地區十字花科蔬菜病毒病害之發生做一調查，並針對重要病原病毒進行媒介昆蟲傳播行為之探討，以作為抗病育種或將來釐訂病害管理策略之參考。

材料與方法

田間病害發生調查

調查台灣南部地區 (台南、高雄、屏東) 十字花科蔬菜栽培地區，各地選擇 5 塊十字花科作物田，每塊田區逢機選取 100 株十字花科蔬菜，以目視法調查作物是否呈現嵌紋、黃化等等不同型態之疑似病毒病病徵及發生率。受測蔬菜種類包括：不結球白菜 (*B. rapa* subsp. *chinensis*)、結球白菜 (*B. rapa* subsp. *pekinensis*)、芥菜類 (*Brassica juncea* var. *strumsta*、*Brassica juncea* var. *oblanceolata*)、甘藍 (*B. oleracea* var. *capitata*)、羽衣甘藍 (*Brassica oleracea* var. *acephala*)、花椰菜 (*B. oleracea* var. *botrytis*)、青花菜 (*B. oleracea* var. *italica*)，以及蘿蔔屬的蘿蔔 (*R. sativus*)。

田間病株病毒種類檢測

進行前項田間病害調查時，就各田區逢機採集 6–10 個疑似病毒病害樣品，摘取新葉 1–2 葉混合，以間接免疫酵素分析法 (indirect enzyme-linked immunosorbent assay; indirect ELISA) 及 6 種病毒抗血清 (BWYV、CaMV、CMV、RaMV、TuMV、TuRSV) 之帶毒率及調查田間不同病毒自然複合感染情形。6 種病毒抗血清來源：TuMV 獲自高雄師範大學退休教授王惠亮博士，CMV 係自行純化病毒免疫紐西蘭大白兔後獲得，CaMV、BWYV、RaMV、TuRSV 等 4 種病毒之抗體免疫球蛋白

皆購自美國 Agdia® (Elkhart, IN, USA)，而正負反應對照亦來自同公司產品。

間接酵素連結免疫分析法：秤取葉片樣品 0.3 g，以 3 mL 之 15 mM 碳酸鈉緩衝液 (sodium carbonate buffer, pH 9.6) 研磨均勻後靜置，上清液加入 ELISA 反應盤內，每個樣品 2 重複，置於 37℃定溫箱進行 4 h 的覆膜反應 (coating reaction)；後以 1× PBST 緩衝液 (137 mM NaCl + 1.5 mM KH_2PO_4 + 1 mM Na_2HPO_4 + 0.05% Tween 20, pH 7.4) 沖洗 3 次；加入病毒抗血清後置於 37℃定溫箱反應 2 h；以 1× PBST 緩衝液沖洗 3 次後，加入以 1× PBST 緩衝液稀釋的黏附鹼性磷酸酵素之山羊抗兔二次抗體 (Alkaline Phsphatase-conjugated AffiniPure Goat anti-Rabbit immunoglobin, Jackson, West Grove, PA, USA) 置於 37℃定溫箱反應 2 h；以 1× PBST 緩衝液沖洗 3 次後，最後加入濃度為 1 mg mL^{-1} 之鹼性磷酸酵素反應基質 (*p*-nitrophenyl phosphate, Sigma, Saint Louis, MS, USA) 進行呈色反應。反應 30–60 min，以 ELISA 判讀儀 (Emax™ Microplate Reader, Molecular Devices, Sunnyvale, CA, USA) 讀取 OD_{405nm} 之吸收值，做為評估病毒濃度高低之依據。樣品讀值大於健康葉片之 2× 者，視為正反應。

檢測種子帶毒率

收集市售十字花科蔬菜：結球白菜 (*B. rapa* subsp. *pekinensis*)、不結球白菜 (*B. rapa* subsp. *chinensis*)、甘藍 (*B. oleracea* var. *capitata*)、油菜 (*Brassica rapa*)、芥菜 (*B. juncea*)、芥藍 (*B. oleracea* var. *alboglaba*)、花椰菜 (*B. oleracea* var. *botrytis*)、青花菜 (*B. oleracea* var. *italica*)、蘿蔔 (*R. sativus*) 共 27 品種 40 批號種子。每批種子隨機檢測 1,000 粒種子 (每 10 粒為一個樣品)，以上述 6 種血清進行檢測，並計算各批種子之帶毒率。

針對重要蚜媒病毒 (TuMV、BWYV) 與媒介昆蟲間關係之探討

將病毒感染株汁液分別接種至奎藜 (*Chenopodium quinoa*) 進行病毒分離，選取單一病斑進行 3 次單斑純化，所得單純病毒材料並以無水氯化鈣進行病毒保存。以單斑分離的病毒株為接種源 (繁殖於油菜或結球白菜)，十字花科葉菜類 (小白菜、葉用蘿蔔) 為被接種材料 (每種作物接種 20 株)，研究田間主要媒介昆蟲：蚜蟲 [偽菜蚜 (turnip aphid)，學名：*L. erysimi* Kaltenbach] 的傳播特性 (獲毒時間、接種時間、帶毒蟲數)。接種後 21 d 觀察植株病徵表現，並配合抗血清進行病毒檢測，測定病毒傳毒率 (以血清及間接 ELISA 檢測全部接種的植株)。無毒蚜蟲取食病毒接種源前，先置於保濕培養皿中經過 24 h 飢餓處理。

蚜蟲數與傳毒效率之關係調查，將無毒棉蚜分別移至 TuMV、BWYV 病毒株上獲毒取食 24 h 後，每一健株分別飼養 0、1、2、5、10 及 20 隻帶毒蚜蟲，經 48 h 後以沾水毛筆尖端移除蟲體，後續觀察病徵表現及測定病毒傳毒率。不同獲毒 (acquisition) 或接種 (inoculation) 取食溫度與傳毒效率之關係調查，將無毒蚜蟲於 15、20、25、30 及 35℃不同溫度下於 TuMV 或 BWYV 病毒株獲毒取食 24 h 後，再移至室溫 (25℃) 2 接種取食健株，48 h 後移除蟲體；或無毒蚜蟲於室溫取食 TuMV 或 BWYV 病毒株獲毒 24 h 後，移至 15、20、25、30 及 35℃不同溫度下取食健株 48 h 後移除蟲體，後續觀察病徵表現及測定病毒傳毒率。

獲毒取食時間與傳毒效率之關係調查，在 TuMV 獲毒取食時間部分，將無毒偽菜蚜分別於 TuMV 病毒株上獲毒取食 0、1、2、5、10、30 及 60 min，再移至健株取食 48 h 後移除蟲體，後續觀察病徵表現及測定病毒傳毒率。BWYV 獲毒取食時間部分，將無毒偽菜蚜分別於 BWYV 病毒株上獲毒取食 0、0.5、1、6、12、24 及 48 h，再移至健株取食 48 h 後移除蟲體，後續觀察病徵表現及測定病毒傳毒率。

接種取食時間與傳毒效率之關係調查，在 TuMV 接種取食時間部分，將無毒偽菜蚜於 TuMV 病毒株上獲毒取食 60 min，再移至健株分別接種取食 0、1、2、5、10、30 及 60 min 後移除蟲體，後續觀察病徵表現及測定病毒傳毒率。在 BWYV 接種取食時間部分，將無毒偽菜蚜於 BWYV 病毒株上獲毒取食 24 h，再移至健株分別接種取食 0、0.5、1、6、12、24

及 48 h 後移除蟲體，後續觀察病徵表現及測定病毒傳毒率。

媒介昆蟲防治效果評估

清潔劑 35% 無患子濃縮液 (soap tree concentrate) 及油類 99% 礦物油 (mineral oil) 等非農藥防治植物保護資材對於蚜蟲的防治效果評估，係以噴壺直接噴灑於飼養 50 隻偽菜蚜的小白菜離葉上 (置於保濕盒中)，分別於 2、4、8、24 及 48 h 後計算蚜蟲致死率，並以噴水為對照，評估其防治效果，每組處理 4 重複。

化學藥劑對於媒介昆蟲防治效果評估，係收集十字花科常用之市售合格推薦殺蚜蟲藥劑 24.7% 賽速洛寧膠囊水懸混劑 [Thiamethoxam + Lambdacyhalothrin suspension concentrates (SC); 61.75 mg a.i. L^{-1}]、16% 可尼丁水溶性粒劑 [Clothianidin soluble granules (SG); 40.00 mg a.i. L^{-1}]、25% 賽速安水溶性粒劑 (Thiamethoxam SG; 50.00 mg a.i. L^{-1})、20% 達特南水溶性粒劑 (Dinotefuran SG; 10.00 mg a.i. L^{-1})、1% 密滅汀乳劑 [Milbemectin emulsifiable concentrate (EC); 6.67 mg a.i. L^{-1}]、20% 亞滅培水溶性粉劑 [Acetamiprid soluble powder (SP); 22.75 mg a.i. L^{-1}]、18.2% 益達胺水懸劑 (Imidacloprid SC; 22.75 mg a.i. L^{-1})、50% 培丹水溶性粉劑 (Cartap SP; 500.00 mg a.i. L^{-1})，並依建議施用倍數稀釋，以噴壺直接噴灑於飼養 50–70 隻偽菜蚜的小白菜離葉上。分別於 2、4、8、24 及 48 h 後計算蚜蟲致死率，並以噴水為對照，評估其防治效果。

結果

調查台灣地區簡易設施十字花科葉菜類栽培種類之病毒發生種類

利用 BWYV、CaMV、CMV、RaMV、TuMV、TuRSV 6 種病毒血清配合間接 ELISA 分析法，進行南部地區簡易設施內十字花科葉菜調查。結果顯示，單一病毒感染情形以 TuMV 感染情形較為嚴重 (7–60%)，BWYV 次之 (0–10%)。二種病毒複合感染以 TuMV 與 BWYV 為主 (0–23%) (表 1)。病毒檢出率高低依序為蘿蔔、芥菜類及白菜類，其中病毒種類以 TuMV 檢出率最高，BWYV 次之；複合感染情形以 BWYV 與 TuMV 居多。

檢測種子帶毒率

以 BWYV、CMV、CaMV、RaMV、TMV 及 TuMV 6 種病毒血清配合間接 ELISA 分析法，進行種子檢測。調查結球白菜 *B. rapa* subsp. *Pekinensis* (「秋陽」、「玉豐」、「綠陽」、「新

表 1. 2018 年 1 月至 2019 年 12 月南部地區十字花科蔬菜單一或二種病毒複合感染情形。

Table 1. Incidence of single or mixed virus infection of field grown cruciferous vegetables detected by indirect enzyme-linked immunosorbent assay (indirect ELISA) in southern Taiwan from January 2018 to December 2019.

Cutivar/species	No. of samples	Virus infection (%)								
		Single virus infection[z]						Mixed virus infection[y]		
		TuMV	BWYV	CMV	CaMV	RaMV	TuRSV	Tu + B	Tu + C	Tu + Ra
Brassica rapa subsp. *chinensis*	164	45	10	0	0	0	0	23	0	0
Brassica rapa subsp. *pekinensis*	136	55	0	0	2	0	0	0	0	0
Brassica juncea var. *strumsta*	56	25	5	0	0	0	0	6	0	0
Brassica juncea var. *oblanceolata*	154	16	0	0	0	0	0	0	0	0
Brassica oleracea var. *capitata*	98	25	8	0	0	0	0	9	0	0
Brassica oleracea var. *acephala*	85	19	3	0	0	0	0	0	0	0
Brassica oleracea var. *botrytis*	20	10	0	0	0	0	0	0	0	0
Brassica oleracea var. *italica*	30	7	0	0	0	0	0	0	0	0
Raphanus sativus	320	43	0	0	0	5	0	0	0	0

[z] TuMV: *Turnip mosaic virus*; BWYV: *Beet western yellows virus*; CMV: *Cucumber mosaic virus*; CaMV: *Cauliflower mosaic virus*; RaMV: *Radish mosaic virus*; TuRSV: *Turnip ring spot virus*.

[y] Tu + B: TuMV + BWYV; Tu + C: TuMV + CMV; Tu + Ra: TuMV + RaMV.

芳 1 號」)、不結球白菜 *B. rapa* subsp. *chinensis* (「三鳳 2 號」、「鳳珍」、「純秀」、「綠愛」)、甘藍 *B. oleracea* var. *capitata* (「夏秋」、「高峰」、「純英」、「春吉」)、油菜 *B. rapa* (「秀春」、「秀珍」、「阿茵」、「阿麗」)、芥菜 *B. juncea* (「青和」、「包心」)、芥藍 *B. oleracea* var. *alboglaba* (「翠津」、「黃花」)、花椰菜 *B. oleracea* var. *botrytis* (「白花 40 」、「夏雲」、「鳳玉」)、青花菜 *B. oleracea* var. *italica* (「清華」、「綠新 3 號」)、蘿蔔 *R. sativus* (「明和」、「白娘」) 共 27 品種 40 批號種子研磨液，經以上述檢測試劑及方法皆未檢測出上述 6 種病毒。

針對重要蚜媒病毒 (TuMV、BWYV) 與媒介昆蟲 (偽菜蚜) 間關係之探討

以 1 隻帶有 TuMV 偽菜蚜餵食葉用蘿蔔 10 d 後，被接種的葉用蘿蔔即可出現葉片嵌紋病徵，傳毒率可達 50%，接種 5 隻帶毒蚜蟲，傳毒率可達 100% (表 2)。蚜蟲獲毒溫度或接種溫度為 25℃時，傳毒率可達 100% (表 3)。蚜蟲獲毒或接種取食時間為 1 min，即可傳播病毒，若蚜蟲獲毒取食時間為 10 min，傳毒率可達 100%。若蚜蟲接種取食時間為 60 min，傳毒率可達 100% (表 4)。

以 1 隻帶有 BYWV 偽菜蚜即可傳毒，5 隻傳毒率則可達 100% (表 2)；當蚜蟲獲毒溫度為 20℃或接種溫度為 25℃時，傳毒率可達 100% (表 3)。蚜蟲取食帶毒植株 1 h 即可獲毒，若取食時間達 48 h，傳毒率可達 100% (表 4)。

表 2.　不同偽菜蚜蟲數對於傳播 TuMV、BWMV 之影響。

Table 2.　Transmission efficiency of TuMV and BWYV by different numbers of *Lipaphis erysimi* Kaltenbach[z].

Number of aphid	Virus transmission efficiency[y] (%)	
	TuMV	BWYV
0	0 (0/10)	0 (0/10)
1	50 (5/10)	40 (4/10)
2	80 (8/10)	70 (7/10)
5	100 (10/10)	100 (10/10)
10	100 (10/10)	100 (10/10)
20	100 (10/10)	100 (10/10)

[z] Aphid transmission was conducted with 24 h acquisition access time followed by 48 h inoculation access time.

[y] TuMV: *Turnip mosaic virus*; BWYV: *Beet western yellows virus*; percentage of virus transmission: (number of plants infected/total number of plant inoculated) × 100%.

媒介昆蟲 (偽菜蚜) 防治效果評估

以非農藥防治資材 (35% 無患子濃縮液及 99% 礦物油) 直接噴灑於飼養偽菜蚜的小白菜離葉上，分別於 2、4、8、24 及 48 h 後計算偽菜蚜致死率。試驗結果為：35% 無患子濃縮液稀釋 50× 之蚜蟲致死率分別為 19.0、30.0、33.5、50.5 及 59.5%；35% 無患子濃縮液稀釋 100× 之蚜蟲致死率，分別為 17.0、26.0、28.0 、48.0 及 56.0%；99% 礦物油稀釋 300× 之蚜蟲致死率，分別為 30.5、35.5、41.0、55.0 及 77.0%；99% 礦物油稀釋 500× 之蚜蟲致死率，分別為 25.0、31.5、36.0、47.0 及 70.5% (表 5)。上述防治資材噴灑後 48 h 對於偽菜蚜防治效果，以 99% 礦物油稀釋 300× 及 35% 無患子濃縮液稀釋 50× 較佳。

利用飼養偽菜蚜的小白菜離葉篩選市面上 8 種常用殺蚜蟲藥劑：24.7% 賽速洛寧膠囊水懸混劑 (61.75 mg a.i. L^{-1})、16% 可尼丁水溶性粒劑 (40.00 mg a.i. L^{-1})、25% 賽速安水溶性粒劑 (50.00 mg a.i. L^{-1})、20% 達特南水溶性粒劑 (10.00 mg a.i. L^{-1})、1% 密滅汀乳劑 (6.67 mg a.i. L^{-1})、20% 亞滅培水溶性粉劑 (33.33 mg a.i. L^{-1})、18.2% 益達胺水懸劑 (22.75 mg a.i. L^{-1})、50% 培丹水溶性粉劑 (500.00 mg a.i. L^{-1})，分別於 2、4、8、24 及 48 h 後計算蚜蟲致死率。試驗結果於藥劑噴灑 48 h 後，蚜蟲致死率均可達 87% 以上 (表 6)。

討論

台灣地區自 1961 年開始研究十字花科作物病毒病至今已近 60 年，當時調查感染蘿蔔之病毒主要有 TuMV、CMV、RaMV 等 3 種。其中，以 TuMV 發生率最高達 96%，CMV 及 RaMV 較少，低於 8% (Kou 1961; Yang 1971)。較近期的田間調查發現十字花科蔬菜病毒病害發生嚴重，極易檢出 TuMV，BWYV 感染率也很高，尤其是芥菜類及蘿蔔高達 52–87 %，顯示此病毒似有寄主偏好性之現象 (Chen *et al.* 2000)。作者於 2018–2019 年調查簡易設施內十字花科蔬菜受病毒感染情形，由結果得

表 **3.** 不同獲毒或接種溫度下取食對偽菜蚜傳播 TuMV、BWMV 效率之影響。

Table 3. Transmission efficiency of TuMV and BWYV by *Lipaphis erysimi* Kaltenbach after treated at various acquisition or inoculation access temperatures.

	Virus transmission efficiency[z] (%)			
	TuMV		BWYV	
Temperature (℃)	Acquisition[y]	Inoculation[x]	Acquisition	Inoculation
15	40 (4/10)	60 (6/10)	90 (9/10)	60 (6/10)
20	80 (8/10)	90 (9/10)	100 (10/10)	90 (9/10)
25	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
30	80 (8/10)	60 (6/10)	40 (4/10)	60 (6/10)
35	20 (2/10)	10 (1/10)	20 (2/10)	10 (1/10)

[z] TuMV: *Turnip mosaic virus*; BWYV: *Beet western yellows virus*; percentage of virus transmission: (number of plants infected/total number of plant inoculated) × 100%.
[y] Turnip aphid acquired virus at different temperatures for 24 h and then inoculated virus at 25℃ for 48 h.
[x] Turnip aphid acquired virus at 25℃ for 24 h and then inoculated virus at different temperature for 48 h.

表 **4.** 不同獲毒或接種時間對於偽菜蚜傳播 TuMV、BWMV 效率之影響。

Table 4. Transmission efficiency of TuMV and BWYV by *Lipaphis erysimi* Kaltenbach after various acquisition or inoculation access time.

	Virus transmission efficiency[z] (%)			
	TuMV		BWYV	
Time	Acquisition[y]	Inoculation[x]	Acquisition	Inoculation
0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
1 min	30 (3/10)	10 (1/10)	-	-
2 min	70 (7/10)	10 (1/10)	-	-
5 min	80 (8/10)	20 (2/10)	-	-
10 min	100 (10/10)	60 (6/10)	-	-
30 min	100 (10/10)	80 (8/10)	0 (0/10)	0 (0/10)
1 h	100 (10/10)	100 (10/10)	20 (2/10)	20 (2/10)
6 h	-	-	20 (2/10)	30 (3/10)
12 h	-	-	40 (4/10)	60 (6/10)
24 h	-	-	70 (7/10)	60 (6/10)
48 h	-	-	100 (10/10)	80 (8/10)

[z] Percentage of virus transmission: (number of plants infected/total number of plant inoculated) ×100%.
[y] Acquisition access time intervals followed by 48 h inoculation access time.
[x] Inoculation access time intervals after 24 h acquisition access time.

表 **5.** 常用非農藥防治資材對於偽菜蚜的致死率。

Table 5. Mortality of turnip aphids by using common non-chemical materials.

		Mortality (%)[z]				
Control material	Dilution (×)	2 h	4 h	8 h	24 h	48 h
35% Soap tree concentrate	50	19.0 ± 2.2	30.0 ± 1.4	33.5 ± 2.2	50.5 ± 1.7	59.5 ± 1.7
35% Soap tree concentrate	100	17.0 ± 2.2	26.0 ± 1.4	28.0 ± 1.4	48.0 ± 1.4	56.0 ± 1.4
99% Mineral oil	300	30.5 ± 1.7	35.5 ± 1.7	41.0 ± 2.2	55.0 ± 2.2	77.0 ± 2.2
99% Mineral oil	500	25.0 ± 2.2	31.5 ± 2.9	36.0 ± 2.8	47.0 ± 2.2	70.5 ± 2.9
CK (H_2O)		4.0 ± 1.4	5.5 ± 0.8	5.5 ± 0.8	5.5 ± 0.8	5.5 ± 0.8

[z] Percentage of mortality: (number of dead turnip apdids/total number of inoculated turnip aphids) × 100%.

表 6. 8 種常用殺蚜蟲藥劑對於偽菜蚜的致死率。

Table 6. Mortality of turnip aphids by using 8 common aphidicides.

Control insecticide[y]	Dilution (×)	Mortality[z] (%) 2 h	4 h	8 h	24 h	48 h
24.7% Thiamethoxam + Lambdacyhalothrin SC	4,000	84.1 ± 3.6	90.8 ± 1.5	96.1 ± 1.8	100.0 ± 0.5	100.0 ± 0.0
16% Clothianidin SG	4,000	63.4 ± 5.3	68.3 ± 2.8	81.4 ± 6.2	91.1 ± 3.6	97.5 ± 0.5
25% Thiamethoxam SG	5,000	66.3 ± 6.2	74.7 ± 5.5	91.6 ± 4.3	96.8 ± 1.6	98.5 ± 1.2
20% Dinotefuran SG	2,000	53.8 ± 4.5	61.9 ± 4.1	71.4 ± 3.9	81.9 ± 3.9	87.6 ± 1.7
1% Milbemectin EC	1,500	67.6 ± 3.5	77.2 ± 2.9	88.1 ± 1.8	94.5 ± 0.8	97.4 ± 0.5
20% Acetamiprid SP	6,000	79.2 ± 2.3	86.3 ± 2.8	94.5 ± 2.7	97.2 ± 2.1	100.0 ± 1.0
18.2% Imidacloprid SC	8,000	75.4 ± 1.6	87.7 ± 1.5	93.8 ± 1.1	96.5 ± 1.3	98.3 ± 0.8
50% Cartap SP	1,000	75.5 ± 1.8	85.8 ± 1.0	97.0 ± 1.5	98.3 ± 0.6	99.1 ± 0.2
CK (H_2O)		1.3 ± 0.1	1.3 ± 0.1	2.6 ± 0.3	6.4 ± 0.2	14.7 ± 1.0

[z] Percentage of mortality: (number of dead turnip apdids/total number of inoculated turnip aphids) × 100%.
[y] SC: suspension concentrates; SG: soluble granules; EC: emulsifiable concentrate; SP: soluble powder.

知白菜類、芥菜及甘藍類以 TuMV 單一感染率最高 (7–55%)，其餘仍以 BWYV 與 TuMV 複合感染居多 (0–23%)。顯示今後在釐訂病害管理措施時，必須 TuMV 及 BWYV 兩者並重。CaMV 為國外常見之十字花科病毒，長久以來在台灣未被發現，直至 Chen *et al.* (2000) 始發現，原因可能與其喜好分布於溫帶氣候及少數幾種蔬菜 (蕪菁 turnip、結球白菜 chinese cabbage) 上，且其發生頻度極低有關 (Farzadfar *et al.* 2004)。在作者本次研究調查的 CaMV 發生率亦低於 2%，且發生的作物為中海拔地區栽培之結球白菜 (*B. rapa* subsp. *pekinensis*)，結果與之相呼應。

十字花科、菊科、禾本科作物或雜草上常見的 5 種蚜蟲，棉蚜 (*Aphis gossypii* Glover)、無肘脈蚜 (*Hysteroneura setariae* Thomas)、偽菜蚜 (*L. erysimi* Kaltenbach)、桃蚜 (*Myzus persicae*)、白尾紅蚜 (*Urolecon formosanus* Takahashi) 中，根據作者 2015–2019 年於高屏地區調查十字花科蔬菜蚜蟲為害種類，其中以偽菜蚜危害為主，故本研究嘗試以偽菜蚜作為 BWYV 及 TuMV 2 種病毒之病毒傳播媒介，用以探討蚜蟲在不同病毒之傳毒行為。由結果顯示，以 1 隻帶 TuMV 蚜蟲餵食健康小白菜、葉用蘿蔔，10 d 後即可出現病徵，同時接種 5 隻帶 TuMV 蚜蟲，傳毒率可達 100%。蚜蟲獲毒溫度或接種溫度為 25℃時，傳毒率皆可達 100%。根據試驗結果，25℃為偽菜蚜獲取 TuMV 及接種時的最適溫度。

以人工接種方式接種 BWYV 分離病毒株為偽菜蚜的獲毒來源，以 1 隻帶有 BYWV 蚜蟲即可傳播病毒，5 隻傳毒效率則可達 100%。當蚜蟲獲毒溫度為 20℃或接種溫度為 25℃時，傳毒效率可達 100%。蚜蟲取食帶毒植株 1 h 即可獲毒，若取食時間達 48 h 以上，傳毒效率可達 100%。BWYV 蚜媒傳播會受溫度影響，隨溫度增高，傳毒率上升，直至 25℃達到 100%，但超過 30℃則明顯下降。結果顯示，偽菜蚜獲取 BWYV 及接種時的最適溫度為 20–25℃。

Gray *et al.* (1991) 曾指出不同蚜蟲種類之 accessory salivary gland 與病毒受體間的親和力有些許差異，其中以桃蚜及偽菜蚜傳毒效率皆可達 100%。又由 BWYV 的蚜蟲傳播試驗結果得知，蚜蟲傳播方式是 BWYV 的病毒傳播特性，並符合 *Luterovirus* 病毒群以循環式 (circulative) 非增殖型 (non-propagative) 方式進行病毒傳播的特性 (Sylvester 1980; Barker & Smith 1999; Hooks & Fereres 2006)。

罹染病毒的十字花科蔬菜目前仍無防治藥劑可供應用，田間管理策略則採用種植健康種苗、注重田間衛生、及時清除病株與周邊其他

寄主，以及適時防治媒介昆蟲為主。本研究利用白菜離葉測試市面上 8 種合格推薦於十字花科使用的常用殺蚜蟲藥劑，顯示 24.7% 賽速洛寧膠囊水懸混劑 (61.75 mg a.i. L^{-1})、16% 可尼丁水溶性粒劑 (40.00 mg a.i. L^{-1})、25% 賽速安水溶性粒劑 (50.00 mg a.i. L^{-1})、20% 達特南水溶性粒劑 (10.00 mg a.i. L^{-1})、1% 密滅汀乳劑 (6.67 mg a.i. L^{-1})、20% 亞滅培水溶性粉劑 (33.33 mg a.i. L^{-1})、18.2% 益達胺水懸劑 (22.75 mg a.i. L^{-1})、50% 培丹水溶性粉劑 (500.00 mg a.i. L^{-1}) 於噴灑 48 h 後，偽菜蚜防治率均可達 87% 以上。以清潔劑及油類等非農藥防治植物保護資材評估對於偽菜蚜的防治效果，以 35% 無患子濃縮液稀釋 50× 及 99% 礦物油稀釋 300× 的蚜蟲防治率較佳。非農藥防治資材的防治效果無法達到立即見效，而是逐步將害蟲族群壓制降到一定密度以下，使生態環境導至到平衡狀態，兼顧農業生產及生態環境的永續性。作者於試驗期間在種子播種或種植後懸掛黃色粘紙誘殺中小型昆蟲，並於播種後 2 葉本葉長出後配合噴施上述合格推薦藥劑，可有效降低簡易網室中蚜蟲數量，降低病毒病害發生率。因此，建議於生長中期可選用中安全容許量之藥劑，愈接近採收期宜選擇、噴施安全採收期短之藥劑，以達到安全用藥的防治策略，可提供農民參考應用。

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Occurrence and Aphid Transmission of Important Virus Diseases in Cruciferous Vegetables

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Abstract

Tsao, L. Y. 2021. Occurrence and aphid transmission of important virus diseases in cruciferous vegetables. J. Taiwan Agric. Res. 70(2):98–107.

Symptoms of suspected virus infection in cruciferous vegetable fields mainly include symptoms of leaf mosaic, mottle, yellows, vein necrosis, vein enation, and stunting. This study used virus sera of *Beet western yellows virus* (BWYV), *Cauliflower mosaic virus* (CaMV), *Cucumber mosaic virus* (CMV), *Radish mosaic virus* (RaMV), *Turnip mosaic virus* (TuMV), *Turnip ring spot virus* (TuRSV) with indirect enzyme-linked immunosorbent assay (indirect ELISA) to investigate the infection ratio of different viruses in the simple facilities. Among them, the detection rate of 6 viruses was the highest with TuMV, followed by BWYV. The most effective mixed-virus infections were BWYV and TuMV. The above 6 sera and indirect ELISA analysis were also used to detect seed grinding liquids of a total of 27 varieties and 40 lots of cruciferous vegetables, including heading Chinese cabbage, non-heading Chinese cabbage, cabbage, rape, mustard, Chinese kale, cauliflower, broccoli and radish. Results showed that no virus was detected. The leaf mustard or leaf radish inoculated with the TuMV single-spot isolated strain or BWYV by artificial inoculation method was used as a source of virus for the turnip aphid (*Lipaphis erysimi* Kaltenbach) to study the transmission characteristics. Symptoms appeared after 10 d of inoculation when a radish leaf was inoculated one turnip aphid with TuMV. The TuMV transmission efficiency can reach to 100% when 5 aphids were inoculated at the same time. When the acquisition and inoculation temperature of turnip aphid was at 25℃, the virus transmission efficiency can reach 100%. The transmission efficiency can reach 100% when the acquisition access time for aphids was 10 min. The virus transmission efficiency reached 100% when the aphid inoculation access time was 60 min. By inoculating BWYV isolates with artificial inoculation as a source of virus for turnip aphid, the efficiency of virus transmission can reach 100% when 5 aphids with BYWV were used to spread the virus. As the acquisition temperature of turnip aphid was set at 20℃, the virus transmission efficiency can reach 100%. When the inoculation temperature was 25℃, the virus transmission efficiency can reach 100%. The transmission efficiency can reach 100% when the acquisition access time was more than 48 h. Using pakchoi leaves to test 8 commonly insecticides found in the market, the aphids control rate can reach 87% or more after spraying 24.7% Thiamethoxam + Lambda-cyhalothrin suspension concentrates (SC), 16% Clothianidin soluble granules (SG), 25% Thiamethoxam SG, 20% Dinotefuran SG, 1% Milbemectin emulsifiable concentrate (EC), 20% Acetamiprid soluble powder (SP), 18.2% Imidacloprid SC, and 50% Cartap SP for 48 h, respectively. Non-pesticide control plant protection materials such as detergents and oils were used to evaluate the control effect on aphids, it showed that the better aphid control rate was found by spraying with 50× of 35% soap tree concentrate and 300× of 99% mineral oil.

Key words: Cruciferous vegetable, *Turnip mosaic virus*, *Beet western yellow virus*, Aphid transmission.

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研究報告

評估取食木油桐 (*Vernicia montana* Loureiro) 花外蜜對基徵草蛉 (*Mallada basalis* Walker) 與安平草蛉 (*Mallada desjardinsi* Navás) 存活之影響

董耀仁[1] 許北辰[1,*]

摘要

董耀仁、許北辰。2021。評估取食木油桐 (*Vernicia montana* Loureiro) 花外蜜對基徵草蛉 (*Mallada basalis* Walker) 與安平草蛉 (*Mallada desjardinsi* Navás) 存活之影響。台灣農業研究 70(2):108–116。

本文呈現取食木油桐花外蜜對基徵草蛉 (*Mallada basalis* Walker) 與安平草蛉 (*Mallada desjardinsi* Navás) 存活影響之試驗結果。其中，花外蜜處理組基徵草蛉與安平草蛉幼蟲較水及空白處理組有顯著較高的存活時間，花外蜜處理組基徵草蛉與安平草蛉成蟲亦有相同情形。取食木油桐花外蜜確實促進基徵草蛉與安平草蛉幼蟲及成蟲具較長壽命，惟無法使基徵草蛉與安平草蛉幼蟲發育及成蟲產卵。取食花外蜜與微膠囊飼料之基徵草蛉與安平草蛉齡幼蟲，對 2 齡棉蚜的捕食能力沒有顯著差異。綜合試驗結果顯示，取食木油桐花外蜜可有效延長基徵草蛉與安平草蛉幼蟲及成蟲存活時間，且不影響幼蟲捕食能力。木油桐有潛力作為基徵草蛉與安平草蛉保育生物防治的應用資材，然仍應在未來實用前先經田間試驗確認其有效性。

關鍵詞：木油桐、花外蜜、基徵草蛉、安平草蛉、保育生物防治。

前言

花外蜜腺 (extrafloral nectaries) 是植物位於花外分泌花外蜜 (extrafloral nectar) 的腺體，目前已知至少有 93 科 332 屬中的植物擁有花外蜜腺 (Jones *et al.* 2017)。花外蜜腺因具有提供植物間接的防禦能力受到學者重視，其所分泌之花外蜜能吸引天敵前來取食同時降低植物遭受植食性昆蟲的危害程度 (Bentley 1977; Heil 2015)。

保育生物防治 (conservation biological control) 策略是一種維持田間天敵族群增強對害蟲生物防治的方式，於田間種植花外蜜腺植物提供天敵所需食物即為其中一種方法 (Simpson *et al.* 2011a, 2011b)。研究顯示花外蜜主要成分為蔗糖、葡萄糖及果糖等單、雙糖與少量胺基酸 (Heil *et al.* 2000)，前人研究中取食花外蜜可以延長天敵壽命、增強尋找寄主/食餌能力、提高寄生與捕食率及增加寄生性天敵子代雌性比例 (Irvin & Hoddle 2015)。

Rezende *et al.* (2014) 於咖啡 (*Coffea* spp.) 園中間種植花外蜜腺植物 *Inga subnuda* subsp. *luschnathiana* 證實可增加天敵數量並降低咖啡潛葉蛾 (*Leucoptera coffeella* Guer) 及咖啡果小蠹 (*Hypothenemus hampei* Ferrai) 對葉片及果實危害。Brown *et al.* (2010) 在蘋果 (*Malus* spp.) 園間種花外蜜腺植物桃樹 (*Prunus* spp.) 降低梨圓盾介殼蟲 (*Quadraspidiotus perniciosus* Comstock) 及椿象 (stink bugs, Pentatomidae) 危害。此外，Xiu *et al.* (2017) 研究指出提供異色瓢蟲 (*Harmonia axyridis* Takizawa) 花外蜜腺植物大麻槿 (*Hibiscus cannabinus* Lin-

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naeus) 含花外蜜的葉片，室內試驗中證實明顯較僅提供水處理組增加壽命，而於田間大麻槿園調查發現即使大麻槿園蚜蟲族群數量低甚至沒有，依然有大量的異色瓢蟲存在於大麻槿園。上述研究顯示，花外蜜腺植物在維持田間天敵族群及增強害蟲生物防治的潛力。

基徵草蛉 (*Mallada basalis* Walker) 與安平草蛉 (*Mallada desjardinsi* Navás) 是屬於脈翅目 (Neuroptera) 草蛉科 (Chrysopidae) 捕食性天敵，草蛉可捕食蚜蟲、粉蝨、薊馬、葉蟎及粉介殼蟲等農業害蟲 (Hsu & Lu 2020)，基徵草蛉幼蟲在台灣可應用在草莓、網室木瓜、棗樹、柑桔等作物防治害蟲，是一種廣譜的捕食性天敵 (Lu & Su 2005)，而安平草蛉亦曾應用於防治桑木蝨 (*Paurocephala psylloptera* Crawford) (Chang 2000)，二者於台灣農業環境中是重要的捕食性天敵 (Lu & Wang 2006; Hsu & Lu 2020)。

木油桐 (*Vernicia montana* Loureiro) 屬落葉性喬木，為森林中優勢木。每年 12 月至隔年 3 月為落葉期及枯葉期，3 月中下旬隨著氣候漸行溫暖而展葉。花多雌雄異株，花期 4 月至 5 月。木油桐葉片心型，常見 3–5 裂，具有長葉柄，頂端有二枚杯狀腺點即花外蜜腺 (So 2004; Lin 2015)。前人研究顯示木油桐害蟲有桑天牛 *Apriona germari* (Hope)、埃及吹綿介殼蟲 *Icerya aegyptiaca* (Douglas)、大避債蛾 *Clania pryeri* (Leech)、綠刺蛾 *Parasa bicolor* (Walker) 及烏桕黃毒蛾 *Euproctis bipunctapex* (Hampson) (Wang *et al.* 2013)。使用蜜源植物進行保育生物防治時，需注意是否蜜源植物成為害蟲食物來源，反而增長害蟲族群發展進而影響天敵之生物防治效果 (Baggen *et al.* 1999)。本研究過程中於戶外觀察木油桐發生害蟲，並未發現上述害蟲有取食木油桐花外蜜情形。迄今，國內尚無花外蜜腺植物與基徵草蛉及安平草蛉之相關試驗研究結果。因此，本試驗將於室內測試取食木油桐花外蜜對基徵草蛉及安平草蛉成蟲及幼蟲之影響。藉由此項研究評估未來利用木油桐等花外蜜腺植物發展草蛉保育生物防治，以提升對害蟲生物防治效果之可行性。

材料與方法

本試驗所使用泥炭土 (Potgrond H, Klasmann-Deilmann GmbH, Geeste, Germany)、木油桐、種植塑膠盆 (直徑 13 cm × 高 12.5 cm)、小黃瓜種植塑膠盆 (直徑 9 cm × 高 6 cm)、透明塑膠培養皿 (直徑 6 cm × 高 1 cm)、玻璃毛細管 (10 μL)、透明壓克力筒 (直徑 15 cm × 高 20 cm) 及產卵紙 (長 50 cm × 寬 15 cm) 均購自振詠興業公司 (台灣台中市)。

本試驗所使用的基徵草蛉 (*Mallada basalis* Walker)、安平草蛉 (*Mallada desjardinsi* Navás)、成蟲飼料、幼蟲微膠囊飼料及相關飼養資材，均來自行政院農業委員會農業試驗所 (以下簡稱農試所) 應用動物組益蟲研究室，草蛉飼育及卵的收集方法參考 Lu & Wang (2006) 的方式。

供試之木油桐 (*Vernicia montana* Loureiro)，係採自農試所園區生長之木油桐植株種子。將種子種植於含泥炭土之塑膠盆中，待木油桐植株生長至葉片花外蜜腺開始分泌花外蜜時，選取含約 15–16 片可分泌花外蜜的木油桐植株 30–50 株，以玻璃毛細管每日收集全株葉片蜜腺所產生之花外蜜，確保收集到足夠可供每日試驗所需花外蜜。

室內試驗之供試小黃瓜植株種植參照 Dong & Hwang (2017) 方法，種植與試驗環境為 25℃ ± 1℃定溫試驗室，光照 12 h (上午 6 h 至下午 6 h)，相對濕度 75% ± 5%。待小黃瓜植株生長至具有 2 片真葉後 (約 14–16 d) 供後續試驗使用。

木油桐對草蛉幼蟲存活時間影響試驗於定溫試驗室內進行，共進行 4 種處理。其中，木油桐處理組為分別將 1–3 齡基徵草蛉或安平草蛉幼蟲 (少於 1 日齡) 1 隻移入內含 0.2 mL 木油桐花外蜜 (前觀察試驗已確認足夠 1 d 取食) 之透明塑膠培養皿中，每日更換含木油桐外源花蜜培養皿，並觀察記錄幼蟲存活時間直至死亡。水處理組試驗方式同上，但透明塑膠培養皿中僅提供水，空白處理組為不提供水及任何食物，微膠囊飼料組為提供草蛉幼蟲微膠囊飼料。本試驗 30 重複。

木油桐對草蛉成蟲存活時間影響試驗於定

溫試驗室進行，共進行 4 種處理。其中，木油桐處理組為將 1 對初羽化 (少於 1 日齡) 之基徵草蛉或安平草蛉成蟲移入內含 0.5 mL 木油桐花外蜜 (先前觀察試驗已確認足夠 1 對草蛉 1 d 取食) 及產卵紙透明壓克力筒中後，每日更換花外蜜及產卵紙，並觀察記錄成蟲存活與產卵數直至成蟲死亡。水處理組試驗方式同上，但壓克力筒中僅提供水，空白處理組為不提供水及任何食物，成蟲飼料組為提供正常草蛉成蟲飼料及水。本試驗 30 重複。

取食木油桐花外蜜對基徵草蛉與安平草蛉幼蟲捕食能力影響試驗參考 Hsu & Lu (2020) 方法，惟於定溫試驗室將取食木油桐花外蜜 (已分別取食 7、14 或 21 d) 或正常室內飼育微膠囊飼料之 1、2 或 3 齡基徵草蛉或安平草蛉幼蟲各 1 隻，分別單獨移入含有 30 隻 2 齡 (少於 1 日齡) 棉蚜的小黃瓜植株上。將草蛉幼蟲移入後，分別經 30 min、1 h、2 h、4 h、8 h 及 24 h 後，觀察記錄小黃瓜植株上草蛉幼蟲取食棉蚜數量。本試驗 30 重複。

試驗所得各項資料 (幼蟲及成蟲存活時間與累計取食棉蚜數量)，先以 SAS-EG (Version 7.1) 統計分析軟體，進行變方分析 (analysis of variance; ANOVA) 後，再以最小顯著差異性測驗 (least significant difference test; LSD test) 分析其差異顯著性。

取食木油桐花外蜜基徵草蛉 1–3 齡幼蟲，顯著較水及空白處理組幼蟲延長存活時間，但無法如取食微膠囊飼料處理組幼蟲完成發育進入下一齡期或化蛹 (表 1)。安平草蛉 1–3 齡幼蟲取食木油桐花外蜜，顯著較水及空白處理組幼蟲延長存活時間，但無法如取食微膠囊飼料幼蟲完成發育進入下一齡期或化蛹 (表 2)。取食木油桐花外蜜基徵草蛉成蟲，顯著較水及空白處理組草蛉成蟲延長存活時間，但基徵草蛉成蟲取食木油桐花外蜜，存活時間顯著少於成蟲飼料處理組且無產卵 (表 3)。取食木油桐花外蜜安平草蛉成蟲，亦顯著較水及空白處理組草蛉成蟲延長存活時間，安平草蛉成蟲取食木油桐花外蜜存活時間顯著少於成蟲飼料處理組且無產卵 (表 4)。

已取食木油桐花外蜜 7、14 及 21 d 基徵草

表 1.　基徵草蛉 (*Mallada basalis* Walker) 幼蟲取食木油桐 (*Vernicia montana* Lour) 花外蜜平均存活時間。

Table 1.　Mean survival time of *Mallada basalis* Walker larvae fed with *Vernicia montana* Lour extrafloral nectar[z].

	Mean survival time (d) (± SE)		
Treatment	First instar	Second instar	Third instar
Blank	1.4 (0.1) c	2.4 (0.1) c	2.3 (0.1) c
Water	1.3 (0.1) c	2.3 (0.1) c	2.4 (0.1) c
Microcapsule diets	3.2 (0.1) b*	3.0 (0.1) b*	4.0 (0.3) b*
Extrafloral nectar	28.6 (1.8) a	29.0 (0.2) a	36.2 (1.8) a

[z] For each column, means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected least significant difference test).

* Already developed to next stage or pupation.

表 2.　安平草蛉 (*Mallada desjardinsi* Navas) 幼蟲取食木油桐 (*Vernicia montana* Lour) 花外蜜平均存活時間。

Table 2.　Mean survival time of *Mallada desjardinsi* Navas larvae fed with *Vernicia montana* Lour extrafloral nectar[z].

	Mean survival time (d) (± SE)		
Treatment	First instar	Second instar	Third instar
Blank	1.5 (0.1) c	2.6 (0.1) c	3.7 (0.3) c
Water	1.4 (0.1) c	2.8 (0.2) c	3.5 (0.3) c
Microcapsule diets	6.8 (0.2) b*	7.7 (0.2) b*	8.1 (0.1) b*
Extrafloral nectar	27.1 (0.8) a	29.1 (2.7) a	34.0 (1.8) a

[z] For each column, means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected least significant difference test).

* Already developed to next stage or pupation.

表3. 基徵草蛉 (*Mallada basalis* Walker) 成蟲取食木油桐 (*Vernicia montana* Lour) 花外蜜平均存活時間及產卵量。

Table 3. Mean survival time and egg production of *Mallada basalis* Walker adult fed with *Vernicia montana* Lour extrafloral nectar[z].

Treatment	Mean survival time (d) (± SE)		Mean cumulative amount of eggs production (± SE)
	Female	Male	
Blank	1.3 (0.1) d	1.3 (0.1) d	0.0 (0.0) b
Water	2.7 (0.1) c	2.7 (0.2) c	0.0 (0.0) b
Extrafloral nectar	27.9 (2.3) b	26.7 (2.2) b	0.0 (0.0) b
Normal adult diets	36.4 (2.2) a	35.2 (2.2) a	415.8 (19.4) a

[z] For each column, means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected least significant difference test).

表 4. 安平草蛉 (*Mallada desjardinsi* Navas) 成蟲取食木油桐 (*Vernicia montana* Lour) 花外蜜平均存活時間及產卵量。

Table 4. Mean survival time and egg production of *Mallada desjardinsi* Navas adult fed with *Vernicia montana* Lour extrafloral nectar[z].

Treatment	Mean survival time (d) (± SE)		Mean cumulative amount of eggs production (± SE)
	Female	Male	
Blank	1.4 (0.1) d	1.1 (0.1) d	0.0 (0.0) b
Water	2.6 (0.1) c	2.4 (0.2) c	0.0 (0.0) b
Extrafloral nectar	26.3 (2.5) b	27.6 (2.1) b	0.0 (0.0) b
Normal adult diet	37.8 (1.8) a	35.7 (2.0) a	273.3 (18.6) a

[z] For each column, means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected least significant difference test).

蛉 1–3 齡幼蟲，於 30 min、1 h、2 h、4 h、8 h 及 24 h 內，累計捕食 2 齡棉蚜的數量與取食微膠囊飼料處理組基徵草蛉 1–3 齡幼蟲無顯著差異 (表 5)。已取食木油桐花外蜜 7、14 及 21 d 安平草蛉 1–3 齡幼蟲，於 30 min、1 h、2 h、4 h、8 h 及 24 h 內，累計捕食 2 齡棉蚜的數量與取食微膠囊飼料處理組安平草蛉 1–3 齡幼蟲無顯著差異 (表 6)。

討論

Limburg & Rosenheim (2001) 研究田間食物對草蛉幼蟲存活之影響，結果顯示試驗中水處理組草蛉 (*Chrysoperla plorabunda* Fitch) 幼蟲平均存活 1.9 d ± 0.1 d，取食棉花花外蜜處理組則平均存活 12.9 d ± 2.0 d。Pfannenstiel & Patt (2012) 研究指出提供黑腳黃囊蜘蛛 (*Cheiracanthium inclusum* Hentz) 水，其平均存活時間為 6.1 d，但當提供棉花花外蜜時，其存活天數可達 52.6 d。本研究中，基徵草蛉與安平草蛉幼蟲取食木油桐花外蜜，亦均較水與空白處理組顯著延長存活時間。而基徵草蛉成蟲與安平草蛉成蟲取食木油桐花外蜜，亦有同樣情形，均顯著較水與空白處理組草蛉成蟲顯著延長存活時間，但仍顯著少於取食正常成蟲飼料處理組。上述試驗結果證實，無正常食餌存在下，取食木油桐花外蜜確能延長基徵草蛉與安平草蛉幼蟲與成蟲存活時間。

Limburg & Rosenheim (2001) 的研究中草蛉 1 齡幼蟲取食棉花花外蜜雖可延長存活時間，但並不能發育至 2 齡。本研究中亦發現，取食微膠囊飼料的基徵草蛉與安平草蛉 1–3 齡幼蟲，能順利發育進入下一齡期或化蛹，但取食木油桐花外蜜之基徵草蛉與安平草蛉 1–3 齡幼蟲，並無法發育至下一齡期或化蛹。此外，取食木油桐花外蜜的基徵草蛉與安平草蛉成蟲，雖能存活但亦無產卵。研究顯示，花外蜜成分組成主要為單、雙糖類的葡萄糖、蔗糖及果糖等醣類與少量的胺基酸，不同具花外蜜腺

表 5. 取食木油桐 (*Vernicia montana* Lour) 花外蜜及微膠囊飼料之基徵草蛉 (*Mallada basalis* Walker) 幼蟲對 2 齡棉蚜 (*Aphis gossypii* Glover) 的捕食能力。

Table 5. Predation capacity of *Mallada basalis* Walker larvae fed on *Vernicia montana* Lour extrafloral nectar and microcapsule diets on second-stage *Aphis gossypii* Glover nymphs[z].

Lacewing larval instar/ treatment	Cumulative number of aphis nymphs consumed by *M. basalis* (± SE)			
		Days after feeding on extrafloral nectar		
	Microcapsule diets	7	14	21
1st/30 min	0.5 (0.1) a	0.7 (0.1) a	0.6 (0.1) a	0.6 (0.1) a
1st/1 h	0.6 (0.1) a	0.7 (0.1) a	0.7 (0.1) a	0.8 (0.1) a
1st/ 2 h	0.9 (0.1) a	0.9 (0.1) a	0.9 (0.1) a	1.1 (0.2) a
1st/ 4 h	2.9 (0.2) a	2.9 (0.2) a	3.1 (0.2) a	3.2 (0.2) a
1st/8 h	6.6 (0.3) a	6.6 (0.4) a	6.7 (0.2) a	6.8 (0.2) a
1st/24 h	15.7 (0.3) a	15.5 (0.4) a	15.7 (0.3) a	16.1 (0.3) a
2nd/30 min	0.6 (0.1) a	0.6 (0.1) a	0.6 (0.1) a	0.6 (0.1) a
2nd/1 h	1.2 (0.1) a	1.2 (0.1) a	1.1 (0.1) a	1.1 (0.1) a
2nd/2 h	1.8 (0.2) a	1.6 (0.1) a	1.7 (0.2) a	1.8 (0.2) a
2nd/4 h	5.0 (0.3) a	5.5 (0.3) a	5.2 (0.3) a	5.1 (0.2) a
2nd/8 h	9.1 (0.3) a	9.5 (0.3) a	9.7 (0.2) a	9.5 (0.3) a
2nd/24 h	23.2 (0.8) a	23.8 (0.7) a	24.1 (0.7) a	24.5 (0.7) a
3rd/30 min	0.6 (0.1) a	0.6 (0.1) a	0.6 (0.1) a	0.7 (0.1) a
3rd/1 h	1.4 (0.1) a	1.4 (0.1) a	1.3 (0.1) a	1.3 (0.1) a
3rd/ 2 h	1.7 (0.1) a	1.7 (0.1) a	1.7 (0.2) a	1.7 (0.1) a
3rd/ 4 h	5.6 (0.3) a	6.0 (0.2) a	6.0 (0.2) a	5.4 (0.2) a
3rd/8 h	10.9 (0.4) a	11.1 (0.4) a	11.3 (0.4) a	11.3 (0.4) a
3rd/24 h	25.7 (0.5) a	26.0 (0.4) a	25.7 (0.6) a	26.6 (0.4) a

[z] For each row, means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected least significant difference test).

植物間花外蜜成分中醣類及胺基酸比例有所差異 (Heil *et al.* 2000; Heil 2015)，食物的不足可能會導致昆蟲發育期延長與生殖力降低 (Morales-Ramos *et al.* 2014; Madadi 2018)，僅取食木油桐花外蜜可能無法提供足夠基徵草蛉與安平草蛉發育與生殖所需營養。

本試驗條件下，已取食木油桐花外蜜經 7、14 及 21 d 之基徵草蛉及安平草蛉 1–3 齡幼蟲，於 30 min 及 1–24 h 內累計捕食 2 齡棉蚜數量與取食微膠囊飼料之草蛉幼蟲並無顯著差異，顯示連續取食木油桐花外蜜對草蛉幼蟲幼蟲捕食害蟲能力似無不利影響。此外，取食木油桐花外蜜之基徵草蛉及安平草蛉 1–3 齡幼蟲，改取食棉蚜後可正常發育進入下一齡期、化蛹及羽化為成蟲 (未發表資料)。顯示先前長期只取食木油桐花外蜜，似未對草蛉幼蟲後來發育造成不利影響。

蘋果園研究發現，距花外蜜植物桃樹 (*Prunus persica* Linnaeus) 盆栽 3 m 範圍內，蘋果樹 (*Malus domestica* Borkh) 上捲葉蚜 (*Aphis spiraecola* Patch) 因受主要捕食性天敵異色瓢蟲 (*Harmonia axyridis* Pallas) 捕食，數量最低。顯示可於田間擺放花外蜜植株盆栽，以達到增強天敵生物防治效果 (Brown & Mathews 2008)。木油桐屬落葉性喬木 (So 2004; Lin 2015)，於田間使用時可參考上述試驗使用木油桐盆栽以便利操作。

保育生物防治藉由採用適當且兼容的田間操作達到維持田間害蟲天敵族群的目的，進而改善並增強生物防治效果 (Madadi 2018)。本

表 6. 取食木油桐 (*Vernicia montana* Lour) 花外蜜及微膠囊飼料之安平草蛉 (*Mallada desjardinsi* Navas) 幼蟲對 2 齡棉蚜 (*Aphis gossypii* Glover) 幼蟲的捕食能力。

Table 6. Predation capacity of *Mallada desjardinsi* Navas larvae fed on *Vernicia montana* Lour extrafloral nectar and microcapsule diets on second-stage *Aphis gossypii* Glover nymphs[z].

Lacewing larval instar/ treatment	Cumulative number of aphis nymphs consumed by *M. desjardinsi* (± SE)			
		Days after feeding on extrafloral nectar		
	Microcapsule diets	7	14	21
1st/30 min	0.6 (0.1) a	0.8 (0.1) a	0.7 (0.1) a	0.7 (0.1) a
1st/1 h	1.1 (0.3) a	1.2 (0.3) a	1.2 (0.3) a	1.3 (0.3) a
1st/ 2 h	1.4 (0.3) a	1.4 (0.3) a	1.4 (0.3) a	1.6 (0.3) a
1st/ 4 h	3.8 (0.4) a	3.7 (0.4) a	3.9 (0.4) a	4.0 (0.4) a
1st/8 h	7.5 (0.4) a	7.5 (0.5) a	7.5 (0.4) a	7.6 (0.4) a
1st/24 h	16.9 (0.5) a	16.6 (0.6) a	16.9 (0.5) a	17.1 (0.5) a
2nd/30 min	0.9 (0.2) a	0.9 (0.2) a	0.9 (0.2) a	0.9 (0.2) a
2nd/1 h	1.5 (0.2) a	1.5 (0.2) a	1.4 (0.2) a	1.4 (0.2) a
2nd/2 h	2.5 (0.4) a	2.3 (0.4) a	2.4 (0.4) a	2.5 (0.4) a
2nd/4 h	5.4 (0.3) a	6.0 (0.3) a	5.7 (0.3) a	5.5 (0.3) a
2nd/8 h	10.0 (0.5) a	10.5 (0.4) a	10.4 (0.4) a	10.4 (0.4) a
2nd/24 h	24.2 (0.7) a	24.6 (0.7) a	24.8 (0.6) a	25.2 (0.6) a
3rd/30 min	0.9 (0.2) a	0.9 (0.2) a	1.0 (0.2) a	1.0 (0.2) a
3rd/1 h	2.1 (0.4) a	2.1 (0.4) a	2.0 (0.4) a	2.0 (0.4) a
3rd/ 2 h	2.8 (0.6) a	2.8 (0.6) a	2.8 (0.6) a	2.8 (0.6) a
3rd/ 4 h	7.0 (0.7) a	7.2 (0.6) a	7.3 (0.6) a	6.7 (0.7) a
3rd/8 h	12.6 (0.9) a	12.8 (0.9) a	13.1 (0.9) a	13.1 (0.9) a
3rd/24 h	26.3 (0.5) a	26.4 (0.4) a	26.1 (0.5) a	27.2 (0.4) a

[z] For each row, means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected least significant difference test).

試驗條件下，取食木油桐花外蜜確可延長基徵草蛉與安平草蛉幼蟲於無正常食餌存在下之存活時間，且取食木油桐花外蜜後並不影響草蛉幼蟲對害蟲的捕食能力。此點有助於田間害蟲族群密度低或無時，仍能維持草蛉族群，以應對後續害蟲發生，達到預防性生物防治效果 (Limburg & Rosenheim 2001; He & Sigsgaard 2019)。惟仍需要詳細評估木油桐花外蜜在三級營養系統中與天敵和害蟲間的相互關係，如應用木油桐花外蜜於田間是否也會成為害蟲食物來源，反而增長害蟲族群發展進而影響天敵之生物防治效果 (Baggen *et al.* 1999)。

雖然於本試驗條件下，取食木油桐花外蜜可延長基徵草蛉與安平草蛉成蟲及幼蟲存活時間，因田間生物與非生物條件複雜，未來若要利用木油桐發展草蛉保育生物防治以提升對害蟲生物防治效果，先於田間試驗確認其可行性是必需的研究。

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Effect of Feeding with Extrafloral Nectar Secretion by Wood Oil Tree (*Vernicia montana* Lour) on Survival of Lacewings, *Mallada basalis* Walker and *Mallada desjardinsi* Navás

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Abstract

Dong, Y. J. and P. C. Hsu. 2021. Effect of feeding with extrafloral nectar secretion by wood oil tree (*Vernicia montana* Lour) on survival of lacewings, *Mallada basalis* Walker and *Mallada desjardinsi* Navás. J. Taiwan Agric. Res. 70(2):108–116.

This study investigated the effects of feeding with wood oil tree (*Vernicia montana* Lour) extrafloral nectar on survival of two lacewings, *Mallada basalis* Walker and *Mallada desjardinsi* Navás. Significant higher survival time of *M. basalis* and *M. desjardinsi* larvae as well as adults were found on the extrafloral nectar treatment than that on the water and blank treatments. Feeding with wood oil tree extrafloral nectar prolonged substantial longevity of *M. basalis* and *M. desjardinsi* larvae and adults. However, consumption of wood oil tree extrafloral nectar did not support the larval development and adult oviposition of both lacewings. Predation capacity on the second-stage of cotton aphid nymphs was not different between extrafloral nectar and microcapsule diet treatments. These results revealed that the extrafloral nectar of wood oil tree could effectively increase larval and adult survival time of *M. basalis* and *M. desjardinsi*, but did not affect larval predation capacity. The wood oil tree may thus be potential as a supplementary tool for conservation biological control program using *M. basalis* and *M. desjardinsi*. However, field tests are necessary to confirm the efficiency of this treatment before further applications.

Key words: *Vernicia montana*, Extrafloral nectar, *Mallada basalis*, *Mallada desjardinsi*, Conservation biological control.

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研究報告

根莖直徑與兩階段連續培養方式對薑組培苗增殖與生長之影響

陳威臣 [1]　曹進義 [2]　吳姿穎 [3]　夏奇鈮 [4,*]

摘要

陳威臣、曹進義、吳姿穎、夏奇鈮。2021。根莖直徑與兩階段連續培養方式對薑組培苗增殖與生長之影響。台灣農業研究 70(2):117–128。

本研究以「廣東薑」(*Zingiber officinale* 'Guang Dong') 與「竹薑」(*Z. officinale* 'Chu') 組織培養根莖進行試驗，探討根莖直徑與液、固態兩階段連續培養方式對組培苗增殖與生長之影響。將直徑分別約 2、4 及 6 mm 根莖接種於含有 1.0 mg L^{-1} 苯甲基腺嘌呤 (benzylaminopurine; BA) 與 0.1 mg L^{-1} 萘乙酸 (α-naphthalene acetic acid; NAA) 之 Murashige & Skoog (MS) 固態培養基培養 8 wk，結果顯示「廣東薑」每一根莖可得 6.2–6.7 芽，但 3 種根莖直徑之間並無顯著差異。「竹薑」4 mm 與 6 mm 之每一根莖可得約 6.8–6.9 芽，顯著高於 2 mm 根莖所得之芽體；同時考慮瓶苗之株高與鮮重表現，則「廣東薑」與「竹薑」瓶苗增殖皆以利用直徑達 4 mm 或以上之根莖較佳。液、固態兩階段連續培養，係先將直徑約 5 mm 之根莖培養於含有 0.1 mg L^{-1} NAA，並分別添加 0、0.5、1.0、2.0 mg L^{-1} BA 之 MS 液態培養基中，培養 2、4 或 6 wk 後，將所得之芽苗團塊直接接種於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 固態培養基中再培養 8 wk。結果顯示，「廣東薑」以含有 2.0 mg L^{-1} BA 經 6 wk 液態培養處理之 24.6 芽顯著最多；「竹薑」在 6 wk 液態培養之 0.5–2.0 mg L^{-1} BA 處理皆具有最高總芽數。綜合上述結果，建議利用直徑 4 mm 以上之根莖作為培植體，可兼顧瓶苗之增殖效率與生長品質；液、固態兩階段連續培養試驗中，若依平均每週可生產之芽數來看，「廣東薑」以 2.0 mg L^{-1} BA 處理與「竹薑」以 1.0–2.0 mg L^{-1} BA 處理之根莖，於液態培養 4 wk 後再繼代培養於固態培養基繼續培養 8 wk，所得之總芽數為最高。

關鍵詞：薑、微體繁殖、組培根莖、液態培養、兩階段連續培養。

前言

薑 (*Zingiber officinale*) 為薑科 (Zingiberaceae) 多年生宿根草本根莖類植物，原生於東南亞熱帶地區，因其根莖 (rhizome) 兼具食用與藥用功能，自古應用於世界各地。薑除了應用在食物調理與藥膳食療外，傳統醫學常用於緩解或治療風濕關節炎、扭傷酸痛、腸胃不適及發燒頭痛等症狀 (Ali *et al.* 2008; Deng & Pan 2009; Rehman *et al.* 2011; Mishra *et al.* 2012; Singh *et al.* 2014)。現代藥理學亦證實，薑具有清熱活血、延緩衰老、護肝保胃及改善血液循環等功能，同時具有降血糖、降血脂和降膽固醇的效果，以及抗菌、抗發炎、抗氧化及抗癌等效用 (Singh *et al.* 2014; Zadeh & Kor 2014; Agrahari *et al.* 2015)。

台灣生薑的主要產區在南投、台東及苗栗等地，種植之品種多為「廣東薑」(*Z. officinale*

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'Guang Dong')，少量為「竹薑」(*Z. officinale* 'Chu')。生薑依其生育期長短，可分為嫩薑、粉薑、老薑 (或稱薑母) 等 3 種 (Agriculture and Food Agency 2017; Council of Agriculture 2017a, 2017b; Chen *et al.* 2018)。薑栽培以無性繁殖根莖為主，但種薑因為常感染薑軟腐病菌 (*Pythium myriotylum*) 與薑青枯病菌 (*Ralstonia solanacearum*) 而品質下降。上述病菌係藉由土壤或種薑而擴大傳播，因此連作薑田之病害發生率高，嚴重影響生薑的產量與品質 (Kambaska & Santilata 2009; Kavyashree 2009; Sathyagowri & Seran 2011)。此外，由於種薑不易長期貯存，栽種適期常面臨種薑缺乏的困境。因此，如何減少種薑帶菌，以及提供足量優質之種薑作為栽培應用，是目前亟需解決的問題。

組織培養技術具有繁殖倍率大、繁殖週期短、可週年生產等優點，乃量產品質均一種苗的有效方法。組織培養種苗繁殖過程係為無菌培養，可排除病原生物之汙染，此優勢是傳統無性繁殖種薑所不能及 (Huang *et al.* 2004; Ma & Gang 2006; Deng & Pan 2009; Lincy & Sasikumar 2010; Liu *et al.* 2010; Seran 2013; Li 2016; Kasilingam *et al.* 2018)。利用液態培養或是液、固態兩階段連續培養方式，可提高組培薑苗增殖與生長效率 (Lincy & Sasikumar 2010; Hapsari *et al.* 2011)。本研究前期已建立「廣東薑」與「竹薑」微體繁殖流程，並以液態培養方式進行大量繁殖，獲得淡黃色叢生芽苗團塊。然而，須將此芽苗團塊移至固態培養基接續培養後，方能長成具有綠色且葉片展開之正常苗，在增殖與養成效率方面仍有改善之空間 (Chen *et al.* 2018)。為提高繁殖效率與瓶苗品質，本研究擬藉由篩選適當培植體根莖直徑與縮短液態培養時間，提升組培苗之增殖與養成效率，達到量產優質種苗之目標。

材料與方法

培植體來源、培養基配製及培養條件

本研究利用 Chen *et al.* (2018) 建立之「廣東薑」('Guang Dong') 與「竹薑」('Chu') 瓶苗為材料。於固態培養基加入 9 g L^{-1} 洋菜 (Bacto Agar, Becton Dickinson and Company, Sparks, MD, USA) 前，先以 0.1–1.0 N NaOH 或 HCl 將 pH 值調為 5.7 ± 0.1，液態培養基則未添加洋菜，且 pH 值調為 5.2 ± 0.1。培養基以 121℃、1.05 kg cm^{-2} 滅菌 20 min 後冷卻備用。固態培養環境為 26℃ ± 2℃、38 μmol m^{-2} s^{-1} 光強度照光 14 h，液態培養條件相同，但光強度為 10 μmol m^{-2} s^{-1}，置於水平迴轉式振盪器 (orbital shaker, SanKuan Co., Taichung, Taiwan) 以 80 rpm 速度進行振盪培養。

根莖直徑對薑組培苗增殖與生長之影響

選取根莖直徑分別約為 2、4 或 6 mm 之「廣東薑」與「竹薑」組培苗，切除根系但保留 2.5 cm 長之假莖作為培植體，接種於內含 100 mL 培養基之 500-mL 蘭花瓶 (orchid flask, Taiwan Glass Ind. Corp., Hsinchu, Taiwan) 中進行試驗。培養基為含有 1.0 mg L^{-1} 苯甲基腺嘌呤 [benzylamino-purine (BA), Sigma, St Louis, MO, USA] 與 0.1 mg L^{-1} 萘乙酸 [α-naphthalene acetic acid (NAA), Sigma, St Louis, MO, USA] 之 Murashige & Skoog (MS) 固態培養基 (Murashige & Skoog 1962)。培養 8 wk 後，調查每一培植體所得之芽數、苗高及鮮重。本試驗每處理採 3 重複，每重複 (瓶) 接種 3 個培植體。

液態培養時間與 BA 濃度對薑組培苗增殖與生長之影響

選取根莖直徑約為 5 mm 之「廣東薑」與「竹薑」組培苗為材料，將切除假莖與根系後之根莖培養於內含 20 mL 培養基之 125-mL 三角瓶 (Erlenmeyer flask) 中進行試驗。根莖培養於含有 0.1 mg L^{-1} NAA 並分別添加 0、0.5、1.0 或 2.0 mg L^{-1} BA 之 MS 液態培養基，於培養 2、4 或 6 wk 後，調查每一培植體所得之芽數。並依芽體生長形態分為葉片未展開之黃色芽 (yellow shoot-bud; YS) 與綠色芽 (green shoot-bud; GS) 及葉片展開芽 (plantlet with unfolded leave; PL) (圖 1)，計算個別數量。將 3 種形態芽體之總和作為總芽數，以各別形態芽體之芽數除以總芽數為其所占比率。本試驗每處理採 3 重複，每重複 (瓶) 接種 3 個培植體。

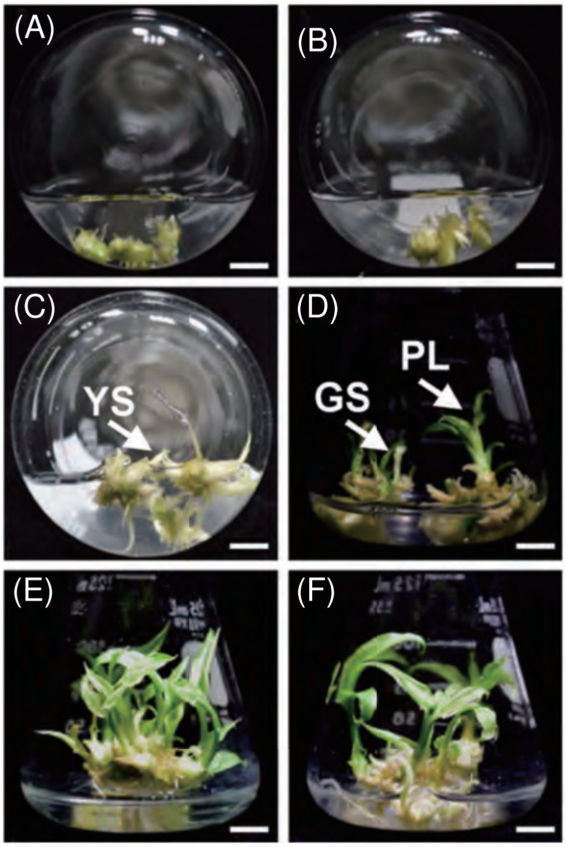

圖 1. 「竹薑」根莖於含有不同濃度 BA 之液態培養基中，培養不同時間後之叢生芽苗團塊或組培苗形成情形。以直徑約 5 mm 根莖培養於含有 0.1 mg L^{-1} 萘乙酸 (α-naphthalene acetic acid; NAA) 之 Murashige & Skoog (MS) 液態培養基，並添加 (A) 0、(B) 1 mg L^{-1} 苯甲基腺嘌呤 (benzyl-aminopurine; BA) 培養 2 wk，(C) 0.5、(D) 2 mg L^{-1} BA 培養 4 wk，以及 (E) 0.5、(F) 1 mg L^{-1} BA 培養 6 wk。YS：黃色芽、GS：綠色芽、PL：葉片展開芽。Bars = 1 cm。

Fig. 1. Shoot-bud clusters and plantlets of *in vitro Zingiber officinale* 'Chu' derived from media with various benzylamino-purine (BA) concentrations and culture durations. *In vitro* rhizomes (about 5 mm in diameter) were cultured in a MS liquid medium supplemented with 0.1 mg L^{-1} NAA and in combined with (A) 0, (B) 1.0 mg L^{-1} BA for 2 wk; (C) 0.5, (D) 2.0 mg L^{-1} BA for 4 wk and (E) 0.5, (F) 1.0 mg L^{-1} BA for 6 wk of culture. YS: yellow shoot-bud; GS: green shoot-bud; and PL: plantlet with unfolded leave. Bars = 1 cm.

液、固態連續培養方式與 BA 濃度對薑組培苗增殖與生長之影響

將上述不同液態培養時間與 BA 濃度之組合試驗所得之芽苗團塊作為培植體，直接接種於內含 100 mL 固態培養基之 500-mL 蘭花瓶中。培養基為含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 固態培養基 (簡稱為生長培養基)，經 8 wk 培養後，調查每一培植體所得之芽數。本試驗每處理採 3 重複，每重複 (瓶) 接種 3 個培植體。

試驗設計和統計分析

本研究採用完全逢機設計 (completely randomized design; CRD) 進行試驗。試驗所得若為百分率資料，則先經角度轉換後再進行分析，經 SAS Enterprise Guide 7.1 套裝統計分析軟體進行變方分析 (analysis of variance; ANOVA)。若處理間差異顯著 ($P < 0.05$)，則以最小顯著差異性測驗 (least significant difference test; LSD test) 比較各處理平均值間之差異。

結果

根莖直徑對薑組培苗增殖與生長之影響

將直徑約為 2、4 或 6 mm 之「廣東薑」與「竹薑」根莖，接種於含有 1.0 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 固態培養基中，培養 8 wk 後之結果如表 1 與圖 2。「廣東薑」每一根莖所得芽數在 6.2–6.7 之間，3 種根莖直徑間並

表 1. 「廣東薑」與「竹薑」根莖直徑對組培苗增殖與生長之影響。

Table 1. Effect of rhizome diameter on proliferation rate and growth of *in vitro Zingiber officinale* 'Guang Dong' and 'Chu' plantlets[z].

Cultivar	Rhizome diameter (mm)	Total plantlets/explant (number)	Plantlet height (cm)	Fresh weight/plantlet (g)
'Guang Dong'	2	6.2 ± 0.3 a[y]	2.6 ± 0.2 b	3.1 ± 0.2 b
	4	6.6 ± 0.1 a	3.0 ± 0.2 ab	4.9 ± 0.3 a
	6	6.7 ± 0.0 a	3.5 ± 0.1 a	5.3 ± 0.1 a
'Chu'	2	4.9 ± 0.2 b	2.5 ± 0.1 b	2.5 ± 0.2 b
	4	6.8 ± 0.3 a	3.2 ± 0.2 a	4.4 ± 0.2 a
	6	6.9 ± 0.1 a	3.1 ± 0.3 ab	4.6 ± 0.5 a

[z] *In vitro* rhizome explants (about 2, 4 or 6 mm in diameter) were cultured on Murashige & Skoog (MS) medium containing 1.0 mg L^{-1} benzyl-aminopurine (BA) and 0.1 mg L^{-1} α-naphthalene acetic acid (NAA) for 8 wk of culture. Each treatment had 3 replications, and 3 explants were used in each replication. Plantlets higher than 1 cm were recorded.

[y] Means in the same column in each cultivar followed by different letters are significantly different at the 5% level by least significant difference (LSD) test.

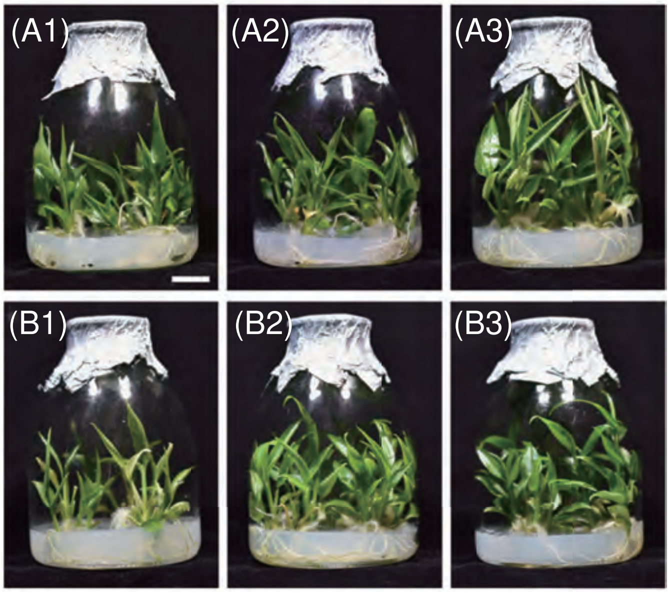

圖 2. 利用不同直徑薑根莖培養所得之組培苗增殖與生長情形。利用根莖直徑約為 (A1、B1) 2 mm、(A2、B2) 4 mm 及 (A3、B3) 6 mm 之 (A1–A3)「廣東薑」與 (B1–B3)「竹薑」根莖培植體，培養於含有 1.0 mg L^{-1} 苯甲基腺嘌呤 (benzyl-aminopurine; BA) 與 0.1 mg L^{-1} 萘乙酸 (α-naphthalene acetic acid; NAA) 之 Murashige & Skoog (MS) 固態培養基中，培養 8 wk 後之組培苗增殖與生長情形。Bar = 2 cm。

Fig. 2. Proliferation and growth of *in vitro Zingiber officinale* plantlets were obtained by using rhizome in different diameters. *In vitro* plantlets of (A1–A3) *Z. officinale* 'Guang Dong' and (B1–B3) 'Chu' derived from rhizomes with about (A1, B1) 2, (A2, B2) 4, and (A3, B3) 6 mm in diameter were cultured on a MS medium supplemented 1.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA for 8 wk of culture. Bar = 2 cm.

無顯著差異。苗高以 6 mm 根莖所得芽體之 3.5 cm 最高，顯著高於 2 mm 根莖所得芽體，但與 4 mm 根莖所得芽體並無顯著差異。鮮重以 4 mm 與 6 mm 根莖所得芽體分別為 4.9 g 與 5.3 g 較重，顯著多於 2 mm 根莖所得芽體。「竹薑」以 4 mm 與 6 mm 之根莖可得約 6.8–6.9 芽，顯著高於 2 mm 根莖所得芽數。苗高以 4 mm 根莖所得芽體之 3.2 cm 最高，顯著高於 2 mm 根莖所得芽體，但與 6 mm 根莖所得芽體無顯著差異。鮮重亦以 4 mm 與 6 mm 根莖所得芽體分別為 4.4 g 與 4.6 g 較重，顯著高於 2 mm 根莖所得芽體。

液、固態兩階段連續培養方式與 BA 濃度對「廣東薑」組培苗增殖與生長之影響

「廣東薑」培植體於不同 BA 濃度之液態培養基中培養不同時間後之變方分析 (ANOVA) 結果顯示，對總芽數而言，液態培養時間呈現非常顯著 ($P < 0.001$) 影響，而 BA 濃度的影響及參試兩因子間的交感效應並不顯著。液態培養時間對 3 種形態芽體之比率，均呈現非常顯著影響；BA 濃度僅對黃色芽 (YS) 比率呈現非常顯著影響，對綠色芽 (GS) 與葉片展開芽 (PL) 比率則具有顯著影響 ($P < 0.05$)。液態培養時間與 BA 濃度之交感效應對葉片展開芽與綠色芽比率，均呈現極顯著 ($P < 0.01$) 差異，但對黃色芽比率則是呈現顯著交感效應 ($P < 0.05$) (表 2)。

「廣東薑」培植體所得總芽數會隨液態培養時間延長而增加，液態培養 2、4 及 6 wk 後之每一培植體分別可得約 3.8–4.6 芽、5.0–5.3 芽及 5.7–6.1 芽。液態培養 2 wk 後，以 2 mg L^{-1} BA 處理之總芽數顯著高於對照組，但與 0.5 或 1.0 mg L^{-1} BA 處理之間並無顯著差異。而液態培養達 4 wk 與 6 wk 後，所有 BA 處理組與對照組間均無顯著差異。對芽體生長狀況而言，在液態培養不同週數後之黃色芽、綠色芽及葉片展開芽 (圖 1) 所占比率均有所差異；當培植體經液態培養 2 wk 後，所得芽體全部為黃色芽；培養 4 wk 後開始產生綠色芽；而葉片展開芽則須在培養達 6 wk 後才會形成，此時對照組之葉片展開芽比率顯著高於 BA 處理組，而 BA 處理組中的 0.5 mg L^{-1} BA 處理顯著高於 2.0 mg L^{-1} BA 處理，但與 1.0 mg L^{-1} BA 處理之間並無顯著差異 (表 2)。

將經由不同液態培養時間與 BA 濃度處理所得之「廣東薑」芽苗團塊，直接接種於固態生長培養基中，經 8 wk 培養後之結果如表 2 顯示。對總芽數而言，液態培養時間與 BA 濃度均呈現非常顯著影響，且參試兩因子間之交感效應也具有顯著差異；液態培養時間對綠色芽與葉片展開芽比率均呈現非常顯著影響，而 BA 濃度對綠色芽與葉片展開芽比率則均無顯著影響；參試兩因子之交感效應對綠色芽與葉片展開芽比率均呈現顯著差異 (表 2)。液態培養 2-wk 處理所得總芽數顯著低於 4-wk 與 6-wk 處理；液態培養 4-wk 之 BA 處理組所得總芽數達 20.0–20.5 芽，顯著高於對照組，其中仍有 10.0–11.7% 為綠色芽；而液態培養 6-wk 之 BA 處理組總芽數亦顯著高於對照組，其中以 2.0 mg L^{-1} BA 處理達 24.6 芽為最高，但其中仍有 13.1% 為綠色芽 (表 2)。

液、固態兩階段連續培養方式與 BA 濃度對「竹薑」組培苗增殖與生長之影響

「竹薑」培植體於不同 BA 濃度之液態培養基中，培養不同時間後之變方分析結果顯示，對總芽數而言，液態培養時間具有非常顯著影響，而 BA 濃度則無顯著影響，且參試兩因子間之交感效應也無顯著差異。液態培養時間在 3 種形態芽體比率上，均呈現非常顯著影響；BA 濃度對黃色芽與綠色芽比率並無顯著影響，但對葉片展開芽比率則具有極顯著影響；液態培養時間與 BA 濃度之交感效應在黃色芽比率上呈現顯著差異，但對於綠色芽比率則無交感效應，而對於葉片展開芽比率則呈現非常顯著交感效應 (表 3)。

「竹薑」培植體所得總芽數亦隨著液態培養時間延長而增加，液態培養 2、4 及 6 wk 後之每一培植體分別可得約 4.0–4.3 芽、5.3–5.9 芽及 5.4–7.0 芽。在液態培養 2 wk 與 4 wk 後，BA 處理組與對照組之間並無顯著差異，而培養達 6 wk 後，則 2.0 mg L^{-1} BA 處理組顯著高於 0.5 mg L^{-1} BA 處理組與對照組，但與 1.0 mg L^{-1} BA 處理組之間並無顯著差異。對組培苗生長狀況而言，在培養 2 wk 後所有芽體仍

表 2. 液、固態兩階段連續培養與 BA 濃度對「廣東薑」組織培養苗增殖與生長之影響。

Table 2. Effects of two-stage continuous culture and BA concentration on proliferation rate and growth of *in vitro Zingiber officinale* 'Guang Dong' plantlets.

		Liquid culture[z]				Solid culture (8 wk)[y]		
Liquid culture period (wk)	BA ($mg\ L^{-1}$)	Total plantlets/ explant	Plantlets with yellowish buds (%)	Plantlets with green shoots (%)	Plantlets with unfolded leaves (%)	Total plantlets/ expla	Plantlets with green shoots (%)	Plantlets with unfolded leaves (%)
2	0.0	3.8 ± 0.2 g[x]	100.0 a	0.0 c	0.0 d	8.9 ± 1.8 e	21.8 ± 6.9 a	78.2 ± 6.9 c
	0.5	4.2 ± 0.3 fg	100.0 a	0.0 c	0.0 d	9.8 ± 1.3 de	19.9 ± 4.6 ab	80.1 ± 4.6 c
	1.0	4.2 ± 0.4 fg	100.0 a	0.0 c	0.0 d	9.8 ± 2.0 de	13.5 ± 2.6 ab	86.5 ± 2.6 bc
	2.0	4.6 ± 0.3 ef	100.0 a	0.0 c	0.0 d	9.7 ± 0.6 de	14.6 ± 5.1 ab	85.4 ± 5.1 bc
4	0.0	5.1 ± 0.2 cde	47.2 ± 16.0 b	52.8 ± 16.0 a	0.0 d	13.0 ± 1.5 c	9.1 ± 4.7 b	90.9 ± 4.7 b
	0.5	5.3 ± 0.1 bcd	65.5 ± 18.6 b	34.5 ± 18.6 ab	0.0 d	20.5 ± 1.3 b	10.1 ± 5.1 ab	89.9 ± 5.1 bc
	1.0	5.2 ± 0.1 bcde	100.0 a	0.0 c	0.0 d	20.1 ± 1.6 b	11.7 ± 2.1 ab	88.3 ± 2.1 bc
	2.0	5.0 ± 0.2 de	100.0 a	0.0 c	0.0 d	20.0 ± 2.8 b	10.0 ± 2.4 ab	90.0 ± 2.4 bc
6	0.0	5.7 ± 0.1 abc	11.8 ± 11.8 c	37.9 ± 4.7 ab	50.3 ± 7.7 a	12.4 ± 1.8 cd	0.0 c	100.0 a
	0.5	6.1 ± 0.4 a	50.2 ± 10.7 b	27.4 ± 10.5 b	22.4 ± 0.4 b	19.6 ± 2.4 b	0.0 c	100.0 a
	1.0	5.8 ± 0.1 ab	50.3 ± 9.2 b	28.9 ± 10.2 ab	20.8 ± 11.3 bc	21.1 ± 1.3 b	12.8 ± 1.6 ab	87.2 ± 1.6 bc
	2.0	5.8 ± 0.2 ab	54.6 ± 19.0 b	38.0 ± 14.4 ab	7.4 ± 4.9 c	24.6 ± 1.7 a	13.1 ± 4.6 ab	86.9 ± 4.6 bc
Source		*F*-test[w]						
Wk		***	***	***	***	***	***	***
BA		ns	***	*	*	***	ns	ns
Wk × BA		ns	*	**	**	***	*	*

[z] *In vitro* rhizome explants (about 5 mm in diameter) were cultured on Murashige & Skoog (MS) liquid medium containing 0.0–2.0 mg L^{-1} benzyl-aminopurine (BA) and 0.1 mg L^{-1} α-naphthalene acetic acid (NAA) for 2, 4 and 6 wk of culture. Each treatment had 3 replications, and 3 explants were used in each replication.

[y] Uncut shoot-bud derived from various treatments in liquid medium were subcultured in the MS solid medium supplemented with 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for extending 8 wk of culture.

[x] Means in each same column followed by different letters are significantly different at the 5% level by least significant difference (LSD) test. Percentage data were arcsine-square-root transformed prior to analysis.

[w] *F*-test of analysis of variance (ANOVA). ns: non-significant; *, ** and ***, are significant at 5%, 1% and 0.1% level, respectively.

表 3. 液、固態兩階段連續培養與 BA 濃度對「竹薑」組織培養苗增殖與生長之影響。

Table 3. Effects of two-stage continuous culture and BA concentration on proliferation rate and growth of *in vitro Zingiber officinale* 'Chu' plantlets.

		Liquid culture[z]				Solid culture (8 wk)[y]		
Liquid culture period (wk)	BA ($mg\ L^{-1}$)	Total plantlets/ explant	Plantlets with yellowish buds (%)	Plantlets with green shoots (%)	Plantlets with unfolded leaves (%)	Total plantlets/ explant	Plantlets with green shoots (%)	Plantlets with unfolded leaves (%)
2	0.0	4.0 ± 0.2 e[x]	100.0 a	0.0 c	0.0 c	4.6 ± 0.1 d	0.0 e	100.0 a
	0.5	4.3 ± 0.0 de	100.0 a	0.0 c	0.0 c	5.4 ± 0.2 cd	9.4 ± 2.3 ab	90.6 ± 2.3 de
	1.0	4.3 ± 0.3 de	100.0 a	0.0 c	0.0 c	6.2 ± 0.6 cd	7.7 ± 2.4 ab	92.3 ± 2.4 de
	2.0	4.2 ± 0.5 e	100.0 a	0.0 c	0.0 c	6.1 ± 1.1 cd	12.9 ± 1.4 a	87.1 ± 1.4 e
4	0.0	5.3 ± 0.2 cd	100.0 a	0.0 c	0.0 c	5.9 ± 0.5 cd	3.2 ± 3.2 cde	96.8 ± 3.2 abc
	0.5	5.8 ± 0.3 bc	92.6 ± 7.4 ab	7.4 ± 7.4 bc	0.0 ± 0.1 c	8.0 ± 1.7 bc	4.7 ± 2.5 bcd	95.3 ± 2.5 bcd
	1.0	5.9 ± 0.1 bc	88.5 ± 8.4 b	11.5 ± 8.4 b	0.0 ± 0.1 c	10.3 ± 1.0 ab	3.2 ± 3.2 cde	96.8 ± 3.2 abc
	2.0	5.9 ± 0.1 bc	87.3 ± 2.3 b	12.7 ± 2.3 ab	0.0 c	11.1 ± 1.2 a	5.4 ± 4.1 abc	94.6 ± 4.1 cde
6	0.0	5.4 ± 0.1 bc	5.9 ± 0.4 d	23.7 ± 5.8 a	70.4 ± 5.8 a	7.2 ± 0.3 cd	0.0 e	100.0 a
	0.5	5.9 ± 0.1 bc	32.2 ± 4.9 c	26.9 ± 3.3 a	40.9 ± 4.2 b	10.4 ± 0.6 ab	1.5 ± 1.1 cde	98.5 ± 1.1 abc
	1.0	6.4 ± 0.2 ab	30.4 ± 8.6 c	25.8 ± 6.4 a	43.8 ± 5.6 b	10.3 ± 0.8 ab	0.0 e	100.0 a
	2.0	7.0 ± 0.6 a	32.0 ± 7.3 c	28.7 ± 2.9 a	39.4 ± 6.8 b	10.7 ± 0.2 ab	0.9 ± 0.9 de	99.1 ± 0.9 ab
Source		*F*-test[w]						
Wk		***	***	***	***	***	***	***
BA		ns	ns	ns	**	***	**	**
Wk × BA		ns	*	ns	***	ns	ns	ns

[z] *In vitro* rhizome explants (about 5 mm in diameter) were cultured on Murashige & Skoog (MS) liquid medium containing 0–2.0 $mg\ L^{-1}$ benzyl-aminopurine (BA) and 0.1 $mg\ L^{-1}$ α-naphthalene acetic acid (NAA) for 2, 4 and 6 wk of culture. Each treatment had 3 replications, and 3 explants were used in each replication.

[y] Uncut shoot-bud derived from various treatments in liquid medium were subcultured in the MS solid medium supplemented with 0.5 $mg\ L^{-1}$ BA and 0.1 $mg\ L^{-1}$ NAA for extending 8 wk of culture.

[x] Means in each same column followed by different letters are significantly different at the 5% level by least significant difference (LSD) test. Percentage data were arcsine-square-root transformed prior to analysis.

[w] *F*-test of analysis of variance (ANOVA). ns: non-significant; *, ** and ***, are significant at 5%, 1% and 0.1% level, respectively.

為黃色芽；而培養達 4 wk 後，BA 處理組約有 7.4–12.7% 綠色芽形成。當培養達 6 wk 後，對照組之黃色芽約為 5.9%，顯著低於 BA 處理組。而在 BA 處理組間並無顯著差異，綠色芽約占 23.7–28.7%，BA 處理組與對照組之間並無顯著差異，此時之對照組已有 70.4% 葉片展開芽，顯著高於 BA 處理組 (表 3)。

將經由不同液態培養時間與 BA 濃度處理所得之「竹薑」芽苗團塊，直接接種於固態生長培養基中，經 8 wk 培養後之結果如表 3 顯示。對總芽數而言，液態培養時間與 BA 濃度均呈現極顯著影響，但兩因子間並無顯著交感效應。液態培養時間對綠色芽與葉片展開芽比率均為極顯著影響；BA 濃度對綠色芽與葉片展開芽比率均為非常顯著影響。參試兩因子間之交感效應，對綠色芽與葉片展開芽比率而言，均無顯著差異 (表 3)。液態培養 2-wk 處理之總芽數，在對照組與 BA 處理組之間並無顯著差異。液態培養 4-wk 處理之 2.0 mg L^{-1} BA 處理組，所得總芽數顯著高於對照組與 0.5 mg L^{-1} BA 處理組，但與 1.0 mg L^{-1} BA 處理組並無顯著差異。液態培養 6-wk 處理之 BA 處理組，所得總芽數約 10.3–10.7 芽，顯著高於對照組，但 BA 處理組之間並無顯著差異，此時所有處理之葉片展開芽比率均已達 98.5–100% (表 3)。

討論

Seran (2013) 評論報告指出，薑根莖培植體大小對組織培養芽體形成與形態發生有顯著影響，較小之培植體因所含養分較少，導致芽體生長較為緩慢。一般而言，薑組培苗之初代培養之適當培植體長度約為 0.5 cm，增殖培養則約在 0.5–2.0 cm 之間。Zuraida *et al.* (2016) 利用 0.5–1.0 cm、2.0–4.0 cm 及大於 4.0 cm 之薑新生芽體進行初代培養試驗，結果以 2.0–4.0 cm 新生芽體培植體所得組培苗數最多。雖然上述兩篇文獻中均未提到新生芽體直徑大小，但是相較而言，較長的新生芽體應當具有較大的根莖直徑。Dal Vesco *et al.* (2001) 於鳳梨 (*Ananas comosus*) 研究結果指出，高度約 3–4 mm 新生芽體培植體相較於 1–2 mm 與 2–3 mm 培植體，對組培苗之生長較為有利。然而，萱草 (*Hemerocallis* spp.) (Chen *et al.* 2005)、海棗 (*Phoenix dactylifera*) (Mazri 2013) 及朝鮮薊 (*Cynara cardunculus*) (El Boullani *et al.* 2017) 研究則指出，組織培養各階段均有其適用培植體大小，過大或太小培植體均不利於組培苗生長。然而，Chen *et al.* (2019) 於孤挺花 (*Hippeastrum hybridum*) 研究結果顯示，培植體來源之鱗莖大小對組培苗增殖並無顯著影響，但對誘導鱗莖之生長具顯著影響，較大培植體能在較短時間內獲得具有較大鱗莖之組培苗。本研究結果顯示，「廣東薑」與「竹薑」組培苗在增殖培養時，其根莖直徑達 4 mm 以上，對芽體之增殖與生長較有利 (表 1)。此結果與 Sathyagowri & Seran (2011) 在薑的研究相符，即較長的新生芽體培植體 (2.0 cm)，應當具有相對較大直徑之根莖，因其內生荷爾蒙或其蓄積之養分充足，相較於較短的根莖培植體 (0.5 cm) 可縮短芽體形成時間，並且促進後續芽體之增殖與生長。本研究與上述研究相較，使用更小之根莖作為繁殖材料，亦能成功快速大量繁殖，此點在繁殖材料短缺時更能發揮其繁殖之效能 (表 1)。

商業化組培苗大量繁殖系統中已有許多重要作物利用液態培養，或是更進一步以反應器進行大規模生產，顯示液態培養方式具有量化繁殖與降低成本的優勢 (Mehrotra *et al.* 2007)。Seran (2013) 評論報告指出，液態培養是促進薑組培苗增殖的有效方法。Mbiyu *et al.* (2012) 於肯亞馬鈴薯 (*Solanum tuberosum*) 研究結果顯示，液態培養所得瓶苗較固態培養者具有較多的莖節與葉片，顯示液態培養對量產組培苗而言，是種高效率的增殖方式。Mehta *et al.* (2014) 於蘋果砧木 (MM106 與 B9) 組培苗研究結果顯示，適當體積 (20 mL) 與短時間 (30 d) 的液態培養相對固態培養之結果，除可增加組培苗之芽數、芽長、葉數、葉面積、根數、根長及出瓶成活率外，培養基成本更可降低 8 倍。

Hapsari *et al.* (2011) 研究結果顯示，以不含生長調節劑且添加食用糖 (table sugar) 之 MS 液態培養基培養 8 wk 後，不僅無損印尼茂物地區 (Bogor area, Indonesia) 紅薑組培苗的生長發育與移植存活率，更有益於降低成

本。然而，Swarnathilaka *et al.* (2016) 以斯里蘭卡薑 (*Z. officinale*, Sri Lanka) 進行微型根莖 (microrhizone) 的研究指出，培植體經 10 wk 液態培養後，微型根莖數量、微型根莖鮮重、組培苗鮮重及根系鮮重均顯著低於固態培養，而在組培苗之數量與株高上則無明顯差異。由此顯示，液態培養並非全然有利於薑組培苗的增殖與生長。Lincy & Sasikumar (2010) 以印度薑 (‘Jamaica’ 與 ‘Varada’) 於 3,000 lux (69 μmol m^{-2} s^{-1}) 條件下進行組培苗研究結果指出，在 1.0 mg L^{-1} thidiazuron (TDZ) 搭配 0.5 mg L^{-1} 或 1.0 mg L^{-1} indolebutyric acid (IBA) 液態培養處理下，雖可提高芽體增殖率，卻形成白色叢生苗，後續將其切割成為含有 4–5 芽之芽苗團塊培植體。培養於固態培養基中 12 d 後，則白色叢生苗開始轉成綠色，培養 45 d 後可形成發根良好的組培苗。顯示利用液、固態兩階段培養方式，具有促進組培苗增殖與生長的效果。Chen *et al.* (2018) 研究結果顯示，「廣東薑」與「竹薑」根莖於液態培養 8 wk 後，將所得之綠色芽或黃色芽繼代培養於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 固態培養基中，經 8 wk 培養後成為葉片展開之正常苗。本研究中液、固態兩階段連續培養試驗結果顯示，液態培養時間與 BA 處理對「廣東薑」與「竹薑」組培苗增殖均具有極顯著影響，而兩因子間交感效應在「廣東薑」為極顯著影響，但在「竹薑」則無顯著影響，顯示兩因子對「廣東薑」與「竹薑」組培苗增殖之影響並不一致 (表 2、表 3)。「廣東薑」總芽數以 2.0 mg L^{-1} BA 處理組在液態培養 6-wk 後再經 8 wk 固態培養處理，顯著高於對照組與其餘 BA 處理組；然而若是以兩階段連續培養之總培養週數作為計算母數，則上述 2.0 mg L^{-1} BA 處理組每週約形成 1.78 芽，明顯高於 2-wk 處理，但與 4-wk 處理結果相近。「竹薑」在上述相同處理條件下，BA 處理組所得總芽數雖然顯著高於對照組，但 BA 處理組之間並無顯著差異。然而，若以每週產芽數作比較，則 2.0 mg L^{-1} BA 處理組每週約形成 0.76 芽，雖明顯高於 2-wk 處理，但卻低於液態培養 4-wk 處理下之 1.0 mg L^{-1} 與 2.0 mg L^{-1} BA 處理組 (表 2、表 3)。因此，若以每週產芽數為準進行比較，則液態培養 4 wk 後再接續於固態培養 8 wk，應為較適當的處理組合。

綜合本研究結果，建議「廣東薑」與「竹薑」組培苗於增殖培養時，根莖直徑至少需達 4 mm 以上；「廣東薑」根莖培養於 2.0 mg L^{-1} BA 處理與「竹薑」根莖培養於 1.0 mg L^{-1} 或 2.0 mg L^{-1} 處理，液態振盪培養達 4 wk 後，將所得芽苗團塊直接繼代培養於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 固態培養基。再經 8 wk 培養後，可得較佳之組培苗增殖與生長效率。上述結果可供薑產業於組培苗量化生產之參考應用。

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Effects of Rhizome Diameter and Two-Stage Continuous Culture on Proliferation Rate and Growth of *In Vitro Zingiber officinale* Plantlets

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Abstract

Chen, U. C., C. Y. Tsao, T. Y. Wu, and C. N. Hsia. 2021. Effects of rhizome diameter and two-stage continuous culture on proliferation rate and growth of *in vitro Zingiber officinale* plantlets. J. Taiwan Agric. Res. 70(2):117–128.

The influence of rhizome diameter and liquid-solid two-stage continuous culture on proliferation rate and growth of *in vitro Zingiber officinale* 'Guang Dong' and 'Chu' plantlets were investigated in this study. The rhizomes of *in vitro* plantlets with about 2, 4 and 6 mm rhizome diameter were cultured on a Murashige & Skoog (MS) solid medium supplemented with 1 mg L^{-1} benzylaminopurine (BA) and 0.1 mg L^{-1} α-naphthalene acetic acid (NAA) for 8 wk of culture. There were 6.2–6.7 plantlets were induced from per explant of 'Guang Dong' rhizome, and no significant difference among three size of rhizomes. There were 6.8–6.9 plantlets were obtained from per 4 mm and 6 mm explants which were significantly higher than that of from 2 mm rhizome of 'Chu'. Moreover, the rhizome diameter larger than 4 mm was considered better explants for growth of the plant height and fresh weight. In the two-stage continuous culture, *in vitro* rhizomes with 5 mm diameter were first culture in a liquid medium containing 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for 2, 4 or 6 wk of culture before subculturing on a solid medium containing 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for 8 wk of culture. The highest number of proliferated plantlets were obtained from the liquid medium containing 2.0 mg L^{-1} BA or 0.5–2.0 mg L^{-1} BA for 6-wk treatment in 'Guang Dong' and 'Chu', respectively. In short conclusion, the diameter of rhizome larger than 4 mm as explants would be beneficial on plantlet proliferation and growth. In according to proliferation efficiency, explants first cultured in a liquid medium containing with 2.0 mg L^{-1} BA in 'Guang Dong' and with 1.0–2.0 mg L^{-1} BA in 'Chu' for 4 wk of culture before subculturing on the solid medium for 8 wk of culture had the highest plantlet yield on a weekly basis of production.

Key word: *Zingiber officinale*, Micropropagation, *In vitro* rhizome, Liquid culture, Two-stage continuous culture.

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研究報告

種子成熟度與播種介質對台灣白及種子瓶外非共生發芽之影響

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摘要

曹進義、陳威臣、吳姿穎、夏奇鈮。2021。種子成熟度與播種介質對台灣白及種子瓶外非共生發芽之影響。台灣農業研究 70(2):129–139。

本研究以自花授粉後 60、90、120、150 及 165 d 之台灣白及 [*Bletilla formosana* (Hayata) Schltr.] 種子，播種於 3 種單一介質，分別為：水苔 (介質 1)、泥炭土 (介質 2)、炭化稻殼 (介質 3)，以及 2 種混合介質配方，分別為砂：壤土：炭化稻殼：珍珠石 = 1 : 1 : 1 : 1 (介質 4) 與砂：炭化稻殼：珍珠石：蛭石 = 1 : 1 : 1 : 1 (介質 5)。5 種介質皆分別經高溫高壓滅菌或不滅菌之處理方式，以探討台灣白及不同成熟度種子在不同介質中於缺乏蘭菌共生情況下 (滅菌介質)，對種子發芽與小苗生長發育之影響。試驗結果顯示，台灣白及在滅菌和未滅菌介質中均可發芽及生長。授粉後 60 d 的種子即具有發芽能力，但以授粉後 165 d 的種子發芽所需時間最短，僅需 9 d。授粉後 90 d 種子之發芽率，在適當培養介質均可達 80% 以上，個別介質中發芽率最高者為滅菌之泥炭土 (84.1%)，其次為滅菌之混合介質 4 (81.1%) 及介質 5 (72.6%)。觀察小苗存活率，在不同授粉日數種子於不同介質中生長之表現並不一致，但以授粉後 120 d 種子在適當介質中皆可達 70% 以上。將授粉後 120 d 種子播種於已滅菌或未滅菌之不同介質中，結果顯示滅菌處理與不同介質對小苗生長發育均呈極顯著影響，且兩者間具交感效應。其中，以經滅菌處理之泥炭土介質為最佳，小苗平均有 3.5 片葉、葉幅為 7.0 mm。本研究之結果顯示，台灣白及具一定成熟度之種子 (60 d)，在適當之培養介質中具有自營發芽與生長之特性。授粉後 120 d 種子在播種後 10 d 即可見種子胚膨大轉綠，17 d 後可見原球體 (protocorm) 分化長出第一片葉，在 120 d 後即可分化成完整植株。介質種類與種子成熟度會影響台灣白及發芽所需時間，而介質滅菌與否對較成熟種子 (≥ 120 d) 的發芽時間影響不顯著。綜此，介質種類、滅菌處理及種子成熟度等因子對種子發芽率與存活率皆有極顯著之影響，且因子間之交感效應顯著。

關鍵詞：蘭花種子、非共生發芽、蘭菌。

前言

台灣白及 [*Bletilla formosana* (Hayata) Schltr.] 為地生類蘭科植物 (terrestrial orchid)，生長地分布於台灣全境之平地與高山地區。台灣白及與中藥材所用之白及為同屬異種，其塊莖亦可作為藥材使用，植株並兼具園藝觀賞價值 (Masuhara & Katsuya 1989)。由於原生地遭受破壞及人為過度採集，台灣白及與中國白及的野生族群皆在快速減少 (Lin *et al.* 1994; Zhu & Wang 1999; Fu *et al.* 2006)，中國並已將白及列為珍稀瀕危的保護植物 (Peng *et al.* 2004)。台灣白及為台灣特有種，有鑑於其族群的快速消失，研究以人工方式大量繁殖其種苗以保護種原之存在有其必要性。根據研究顯示，蘭科植物成熟種子非常細小，單粒重約 0.3–14.0 μg，長 0.25–1.20 mm，外披透明

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單層細胞之種皮，細胞內容物已消失，胚亦只發育到圓球胚 (globular embryo) 階段即停止，不具胚乳亦無子葉、胚根及胚芽等組織 (Lee 1990)。蘭科植物之種子因發育不全，於自然狀態下其種子發芽需藉由與菌根菌 (mycorrhiza) 共生取得養分，才能發芽進而發育成正常之幼苗 (Arditti 1966, 1967; Yamamoto *et al.* 2017)。此外，利用植物組織培養技術進行蘭科植物無菌播種，以培養基中的營養成分取代菌根菌的功用，則是蘭花人工繁殖上很大的突破 (Juang & Lee 1985)。本研究團隊過去利用無菌播種與試管內培養方式，已成功改進台灣白及種子發芽與小苗之生長 (Tsao *et al.* 2011)，但仍思考是否有更簡單易行之人工繁殖方式可作為台灣白及就地復育之用。由於觀察到少數幾種人工栽培之地生蘭如台灣白及、綬草屬 (*Spiranthes*) 及苞舌蘭屬 (*Spathoglottis*) 之種子，具有自行發芽並長成小苗之現象。為證明此一觀點應用於台灣白及之可行性及其所需之條件，本研究將台灣白及不同成熟度種子播種於滅菌或未滅菌之不同介質中，觀察種子成熟度與介質種類對種子發芽與小苗生長之影響。本研究之目的，除欲證實蘭科植物可以不經由與菌根菌共生而正常發芽生長外，更期望此一簡單之人工大量繁殖方式能幫助台灣白及免除種原滅絕危機。

材料與方法

試驗材料

本研究使用之台灣白及採集自新北市平溪地區，植株種植於行政院農業委員會農業試驗所溫室內，開花後進行人工自花授粉。種子成熟度依授粉後天數區分，分別為 60、90、120、150 及 165 d，其中 120 d 果莢已轉黃，150 d 及 165 d 之果莢已開裂。播種時，取上述授粉後不同天數之種子作為播種材料。

播種介質與酸鹼值檢測

播種用介質分別為單一介質之水苔 (介質 1)、泥炭土 (介質 2)、炭化稻殼 (介質 3)，以及混合介質，其配方分別為砂：壤土：炭化稻殼：珍珠石 (粒徑 2–5 mm) (體積比 = 1 : 1 : 1 : 1) (介質 4) 及砂：炭化稻殼：珍珠石：蛭石 (粒徑 5–6 mm) (體積比 = 1 : 1 : 1 : 1) (介質 5)，介質中之砂與壤土取自農試所田區。播種介質以 121℃、1.21 kg cm^{-2} 進行二次 40 min 的高溫高壓滅菌。將滅菌或未滅菌介質平鋪於直徑 90 mm、高度 15 mm 的無菌透明塑膠培養皿內備用。以酸鹼檢測儀 (S20 SevenEasy^{TM} pH, Mettler Toledo, Zürich, Switzerland) 檢測滅菌或未滅菌介質之酸鹼值，取樣方式為分別逢機取 3 g 介質，加入 100 mL 去離子水，浸泡 24 h 後進行 pH 值檢測 (Chang *et al.* 2006)。

種子播種與小苗移植

由於台灣白及種子外觀較其他蘭花種子大且明顯，容易藉由肉眼挑選較均質一致的種子，因此以人工分別挑選外觀飽滿完整與大小趨近一致較均質之 5 種不同授粉天數種子，分別播種於上述 5 種介質中。每一培養皿皆均勻播種 300 粒種子為 1 重複，共進行 4 重複。將加蓋之培養皿放置於農業試驗所生技組具風扇水牆之環控溫室內，播種後水分管理以滅菌之去離子水以塑膠噴壺水霧噴濕，每隔 3 d 噴水 1 次。培養期間除噴霧加濕時掀開上蓋，其餘時間均保持蓋上之狀態。播種後，以每皿 50 粒種子以上發芽，則調查其起始發芽天數。種子發芽以長出第一片葉作為標準，於播種 60 d 後調查發芽率，而於 120 d 後計算小苗存活率。因授粉後 120 d 種子已有良好之發芽能力，同時評估其發芽天數、發芽率以及存活率。因此，在另一個試驗以授粉後 120 d 之種子播種於已滅菌之 5 種介質中，每一培養皿均勻播種 300 粒種子為 1 重複，共進行 4 重複。分別於播種後 60 d 與 120 d 後，調查小苗之葉數與葉幅，之後將存活之小苗單株移植至盛裝有泥炭土介質 (PEAT MOSS 7-1, Bas van Buuren B.V., De Lier, The Netherlands) 之 72 孔穴盤定植。

統計分析

試驗採用完全逢機設計 (completely randomized design; CRD)，試驗資料先進行變方分析 (analysis of variance; ANOVA)。若處理間差異顯著，再進行最小顯著差異性測驗 (least significant difference; LSD) 比較各處理平均值

間的差異性。其中，發芽率與存活率之數據先經 $\sin^{-1}$ 角度轉換後，再進行統計分析。各項分析工作利用 SAS Enterprise Guide 7.1 統計分析套裝軟體進行。

結果

不同授粉後天數種子與播種介質對於種子發芽之影響

比較 3 種單一介質 (介質 1、介質 2、介質 3) 及 2 種混合介質 (介質 4、介質 5) 之已滅菌與未滅菌介質之 pH 值，結果顯示相同介質在未滅菌與滅菌處理後其 pH 值之差異不大，其中水苔、泥炭土為弱酸性，pH 分別為 4.36–4.92 與 5.90–6.03，而炭化稻殼及混合介質 4 與介質 5 為中性，pH 為 6.86–7.42 (表 1)。

表 2 顯示台灣白及不同授粉後天數種子於 5 種介質中發芽所需之最少天數，其中介質是否滅菌對發芽天數之影響多不顯著，僅授粉後 90 d 之種子在介質 3、介質 4 及介質 5 中有較明顯差異，但皆以未滅菌介質中之發芽天數較低。此外，在授粉後 60 d 種子即有發芽能力，但發芽僅見於介質 2，於其他 4 種介質中皆未見種子發芽。授粉 90 d 後之種子，可於所有介質中發芽。觀察同一介質中隨著種子之授粉後天數增加，種子發芽所需時間有縮短之趨勢，以授粉後 165 d (果莢已自然黃熟裂開) 之種子所需發芽天數最短，僅需 9 d；但其中授粉後 150 d 之發芽天數較為偏高。觀察相同授粉後天數之種子在不同介質中之發芽天數，以介質 2 所需天數較短，在 9–21 d 之間 (表 2)。

表 1. 台灣白及播種所使用未滅菌及經高溫滅菌之 5 種介質酸鹼值。

Table 1. Effect of an autoclave on the changes of pH value in 5 growth media used for germination test of *Bletilla formosana* (Hayata) Schltr. seeds.

Medium No.[z]	pH	
	Non-autoclaved	Autoclaved
1	4.92	4.36
2	5.90	6.03
3	7.42	7.21
4	7.21	7.23
5	6.96	6.86

[z] Media from 1 to 5 were sphagnum moss, peat moss, carbonized rice husk, sand : loam : carbonized rice husk : perlite = 1 : 1 : 1 : 1 (v/v), and sand : carbonized rice husk : perlite : vermiculite = 1 : 1 : 1 : 1 (v/v), respectively.

表 2. 台灣白及不同授粉後天數種子於滅菌或未滅菌 5 種介質中對發芽天數之影響。

Table 2. Effects of seed maturity, growth media composition and sterilization of growth media on seed germination of *Bletilla formosana* (Hayata) Schltr.[z]

Media	Autoclave	Seed maturity (d)[y]				
		60	90	120	150	165
1	No	-[x]	43_{d}^{D}[w]	30_{c}^{B}	34_{c}^{C}	23_{c}^{A}
1	Yes	-	43_{d}^{D}	30_{c}^{B}	34_{c}^{C}	23_{c}^{A}
2	No	16_{a}^{B}	21_{a}^{C}	10_{a}^{A}	19_{a}^{C}	9_{a}^{A}
2	Yes	16_{a}^{B}	21_{a}^{C}	10_{a}^{A}	19_{a}^{C}	9_{a}^{A}
3	No	-	28_{b}^{B}	37_{d}^{D}	34_{c}^{C}	16_{b}^{A}
3	Yes	-	43_{d}^{D}	37_{d}^{C}	34_{c}^{B}	16_{b}^{A}
4	No	-	21_{a}^{C}	16_{b}^{B}	26_{b}^{D}	9_{a}^{A}
4	Yes	-	35_{c}^{D}	16_{b}^{B}	26_{b}^{C}	9_{a}^{A}
5	No	-	21_{a}^{C}	16_{b}^{B}	19_{a}^{C}	9_{a}^{A}
5	Yes	-	28_{b}^{D}	16_{b}^{B}	19_{a}^{C}	9_{a}^{A}

[z] Each treatment had 4 replications and each replication contained 300 seeds in a petri dish. Germination day was recorded while more than 50 seeds germinated in a petri dish.

[y] Seed maturity was days after pollinating.

[x] -: no seed germination.

[w] Means in the same column with different subscript letters or A, B, C, D in the same row with different capital letters are significantly different at $P < 0.05$ by least significant difference (LSD) test.

介質滅菌處理、不同授粉後天數種子及介質種類對於種子發芽率與小苗存活率之影響

介質是否滅菌處理、不同授粉後天數種子、介質種類 3 個單因子、兩兩單因子間及 3 個因子間之交感，皆顯著影響種子發芽率與小苗存活率。滅菌處理對發芽造成之影響在介質 2 和介質 4 較明顯，而在介質 1 之差異最不明顯 (表 3)。觀察滅菌處理對小苗存活之影響，則以介質 4 之差異最明顯，而以介質 1 最不明顯。綜觀同一授粉後天數之種子在相同介質中之發芽率及存活率，滅菌或未滅菌處理大都在統計上有相似之結果。若有顯著差異者，則大都以滅菌介質表現較未滅菌處理為佳。比較相同授粉後天數種子在不同介質中之發芽率，大致以滅菌之介質 2、介質 4 及介質 5 較高；所有介質中除介質 3 之存活率較差外，其餘介質對存活率之影響表現並不一致；但針對不同授粉後天數種子，則以介質 4 之整體表現最佳。觀察不同授粉後天數種子之表現，顯示授粉後 60 d 種子只在滅菌介質 2 具發芽率，但授粉後 90 d 種子則在不同介質中均能發芽，但以授粉後 120 d 之後的種子發芽率明顯提高。整體而言，除少數介質如滅菌介質 4 與未滅菌介質 2 組外，皆以授粉後 165 d 之種子有較高之發芽

表 3. 台灣白及不同授粉後天數種子於 5 種介質中對發芽與小苗存活之影響。

Table 3. Effects of seed maturity and growth media composition on asymbiotic seed germination and survival rates of *Bletilla formosana* (Hayata) Schltr.

		Germination rate (%)[y]					Survival rate (%)[x]				
Media[z]	Autoclave	60 d	90 d	120 d	150 d	165 d	60 d	90 d	120 d	150 d	165 d[w]
1	No	$0.0_{c}^{C\ v}$	34.4_{cd}^{B}	42.8_{e}^{AB}	45.8_{c}^{A}	42.1_{d}^{AB}	-[u]	30.1_{ab}^{B}	15.3_{cde}^{C}	42.7_{c}^{A}	32.5_{b}^{B}
1	Yes	0.0_{c}^{C}	34.4_{cd}^{B}	40.3_{e}^{AB}	43.8_{c}^{A}	40.1_{d}^{AB}	-	30.0_{ab}^{B}	14.1_{cde}^{C}	40.7_{c}^{A}	31.5_{b}^{B}
2	No	19.5_{b}^{E}	27.9_{d}^{D}	71.8_{bc}^{A}	61.5_{b}^{B}	42.8_{d}^{C}	0.0^{B}	2.2_{d}^{B}	29.2_{bc}^{A}	7.0_{d}^{B}	11.8_{cd}^{B}
2	Yes	38.8_{a}^{C}	75.8_{b}^{B}	90.0_{a}^{A}	84.4_{a}^{AB}	86.2_{ab}^{AB}	0.0^{D}	6.0_{d}^{D}	73.2_{a}^{A}	43.1_{c}^{B}	16.3_{cd}^{C}
3	No	0.0_{c}^{D}	33.0_{cd}^{C}	52.8_{de}^{B}	27.9_{d}^{C}	80.9_{b}^{A}	-	16.0_{cd}^{B}	4.1_{de}^{B}	14.2_{d}^{B}	39.6_{b}^{A}
3	Yes	0.0_{c}^{D}	44.1_{c}^{B}	43.8_{e}^{B}	20.4_{d}^{C}	61.9_{c}^{A}	-	11.3_{cd}^{B}	0.7_{e}^{C}	14.2_{d}^{B}	23.3_{cd}^{A}
4	No	0.0_{c}^{E}	35.2_{cd}^{D}	62.2_{cd}^{B}	49.1_{bc}^{C}	82.4_{b}^{A}	-	3.6_{d}^{B}	0.0_{e}^{B}	6.5_{d}^{B}	40.4_{b}^{A}
4	Yes	0.0_{c}^{D}	95.5_{a}^{A}	69.8_{bc}^{C}	75.8_{a}^{B}	83.2_{ab}^{B}	-	31.3_{a}^{B}	38.8_{b}^{B}	61.4_{ab}^{A}	77.1_{a}^{A}
5	No	0.0_{c}^{C}	25.8_{d}^{B}	70.6_{bc}^{A}	79.6_{a}^{A}	60.9_{c}^{A}	-	16.7_{bcd}^{B}	22.0_{bcd}^{AB}	45.4_{bc}^{A}	45.2_{b}^{A}
5	Yes	0.0_{c}^{C}	40.4_{c}^{B}	80.1_{ab}^{A}	76.5_{a}^{A}	93.2_{a}^{A}	-	23.4_{abc}^{B}	14.6_{cde}^{BC}	71.8_{a}^{A}	6.6_{d}^{BC}
Significance			Germination (%)		Survival (%)						
Day (D)			** [t]		**						
Media (Me)			**		**						
Autoclave (A)			**		**						
D × Me			**		**						
A × Me			**		**						
A × D			**		**						
D × Me × A			**		**						

[z] Media from 1 to 5 were sphagnum moss, peat moss, carbonized rice husk, sand : loam : carbonized rice husk : perlite = 1 : 1 : 1 : 1 (v/v), and sand : carbonized rice husk : perlite : vermiculite = 1 : 1 : 1 : 1 (v/v), respectively.

[y] Germination rates were recorded 60 d after sowing.

[x] Survival rates were recorded 120 d after sowing.

[w] Seed maturity were days after pollinating.

[v] Means in the same column with different subscript letters or A, B, C, D, E in the same row with different capital letters are significantly different at $P < 0.05$ by least significant difference (LSD) test.

[u] -: no seed Germination.

[t] **: significant at 0.01 level.

率。不同授粉後天數之種子在同一介質中之存活率表現較不一致，如授粉後 150 d 種子之最佳存活率為介質 5，授粉後 120 d 種子則以介質 2 為最高，授粉後 165 d 種子則以介質 3、介質 4 及介質 5 (未滅菌) 為最高。綜合結果顯示，授粉後 60 d 之種子播種於已滅菌之介質 2 即具發芽能力，但於播種後 120 d 調查小苗之存活率皆為 0%。授粉後 90 d 以上之種子播種於 5 種介質中皆可發芽，但於滅菌之介質 4 中有最高之發芽率為 95.5%，但其存活率僅 38.8%。授粉後 120 d 之種子播種於滅菌介質 2 有高發芽率及存活率，分別為 90.0% 與 73.2%。授粉後 150 d 與 165 d 果莢之種子分別播種於滅菌之介質 4 與介質 5，發芽率分別為 75.8% 和 93.2%，存活率除授粉後 165 d 種子於滅菌介質 5 之 6.6% 較低外，其餘存活率在 61.4–77.8% 之間 (表 3)。綜合發芽率與存活率來看，以授粉後 120 d 之種子播種於滅菌介質 2 為最佳，發芽率與存活率在不同授粉天數種子則以滅菌之介質 4 表現較為一致。

以授粉後 120 d 種子播種於 5 種介質在生長 120 d 後觀察小苗於滅菌或未滅菌不同介質中生長之情形，結果顯示以滅菌之介質 2 對小苗生長與發育為最佳，平均葉數為 3.5 片葉，雙葉幅可達 7.0 mm，而以介質 1 與介質 3 為最差，小苗生長與發育極為緩慢 (表 4、圖 1)。本試驗以授粉後 120 d 果莢之種子播種於滅菌介質 2 為模式，觀察台灣白及種子發芽與發育之過程顯示，在播種後第 10 天，種子胚已膨大轉綠；在第 17 天時，原球體 (protocorm) 開始分化長出第一片葉；在第 31 天時，第一片葉分化生長完成；在第 60 天時，長出第二片葉；在第 120 天時，開始長出第三片葉與根發育成完整植株 (圖 2)。此外，觀察小苗生長過程中發現，小苗易遭受蟲害啃食與青苔等綠色黏稠狀物質覆蓋 (未顯示)，因而影響後期小苗生長與存活之情形，將已存活之小苗個別移植至已滅菌之泥炭土介質，小苗可繼續正常生長發育 (圖 3)。

討論

蘭科植物種子非常細小，通常只發育至圓

表 4. 不同介質對台灣白及種子小苗生長之影響。

Table 4. Effects of growth media composition on the growth of *Bletilla formosana* (Hayata) Schltr. seedlings[z].

		60 d after sowing		120 d after sowing	
Medium	Autoclave	Leaf number	Leaf span (mm)	Leaf number	Leaf span (mm)
1	No	1.0 c[y]	0.9 d	1.2 c	1.2 cd
1	Yes	1.0 c	0.8 d	1.2 c	1.3 cd
2	No	1.3 b	2.3 b	2.0 b	3.2 b
2	Yes	1.9 a	2.9 a	3.5 a	7.0 a
3	No	1.0 c	0.7 de	1.0 c	0.7 d
3	Yes	1.0 c	0.6 e	1.0 c	0.7 d
4	No	1.0 c	1.0 d	-[x]	-
4	Yes	1.1 c	1.4 c	2.0 b	1.8 cd
5	No	1.1 c	1.5 c	2.0 b	3.2 b
5	Yes	1.1 c	1.6 c	2.0 b	2.3 bc
Significance					
Media (Me)		** [w]	**	**	**
Autoclave (A)		**	**	**	**
A × Me		**	**	*	**

[z] Seeds were taken from the capsule which was 120 d after pollination.
[y] Means in the same column with different letters are significantly different ($P < 0.05$) by least significant difference (LSD) test.
[x] -: no seedling survived.
[w] *, **: significant at 0.05 and 0.01 level, respectively.

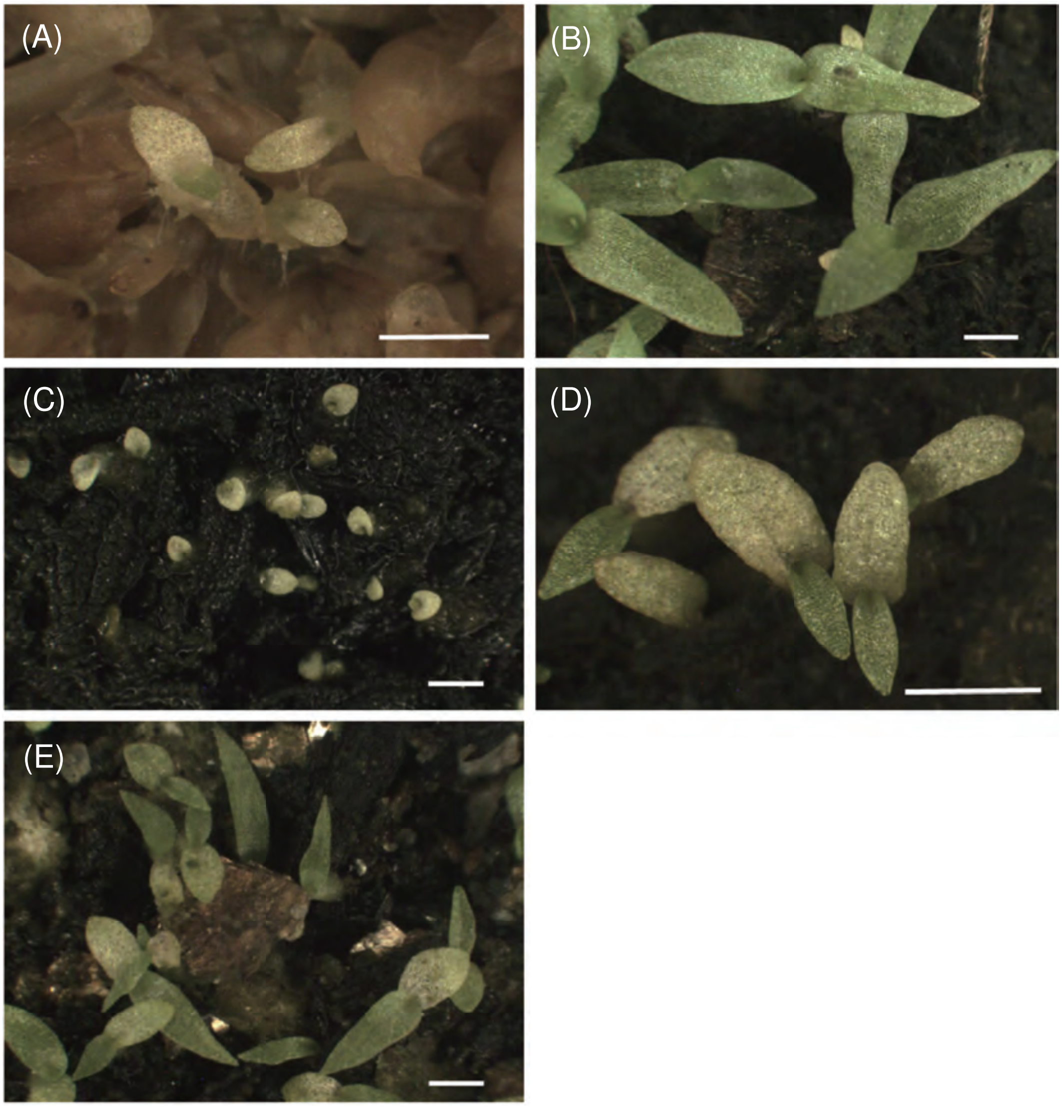

圖 1. 以授粉後 120 d 之台灣白及種子播種於已滅菌處理之 (A) 水苔；(B) 泥炭土；(C) 炭化稻殼；(D) 砂：壤土：炭化稻殼：珍珠石 (1 : 1 : 1 : 1) 混合介質；(E) 砂：炭化稻殼：珍珠石：蛭石 (1 : 1 : 1 : 1) 混合介質，在播種後 120 d 小苗之生長情形。Bar = 1 mm。

Fig. 1. Growth of *Bletilla formosana* (Hayata) Schltr. seedlings on different compositions of growth media after asymbiotic germination from seeds collected 120 days after pollination (DAP). Growth medium of (A) sphagnum moss; (B) peat moss; (C) carbonized rice husk; (D) sand : loam : carbonized rice husk : perlite = 1 : 1 : 1 : 1; (E) sand : carbonized rice husk : perlite : vermiculite = 1 : 1 : 1 : 1. Bar = 1 mm.

球胚期即停止發育，屬於未成熟胚，於野生狀態下必需藉由菌根菌 (mycorrhiza) 提供種子發芽養分才能繼續發育長成小苗 (Arditti 1966, 1967; Yamamoto *et al.* 2017)。過去的研究顯示，蘭科植物種子之發芽及後續發育可藉由人工接種菌根菌，藉由共生菌幫助種子水分與養分之吸收並促進苗株生長 (Lin 2002; Kang 2004; Rasmussen *et al.* 2015; Alghamdi 2019; Yeh *et al.* 2019)。目前有關蘭科植物不藉由菌根菌共生或組織培養方式，其種子仍可正常發芽並完成後續發育之相關研究甚少。Su (1996) 研究顯示，於台灣白及生育地取樣土壤，進行

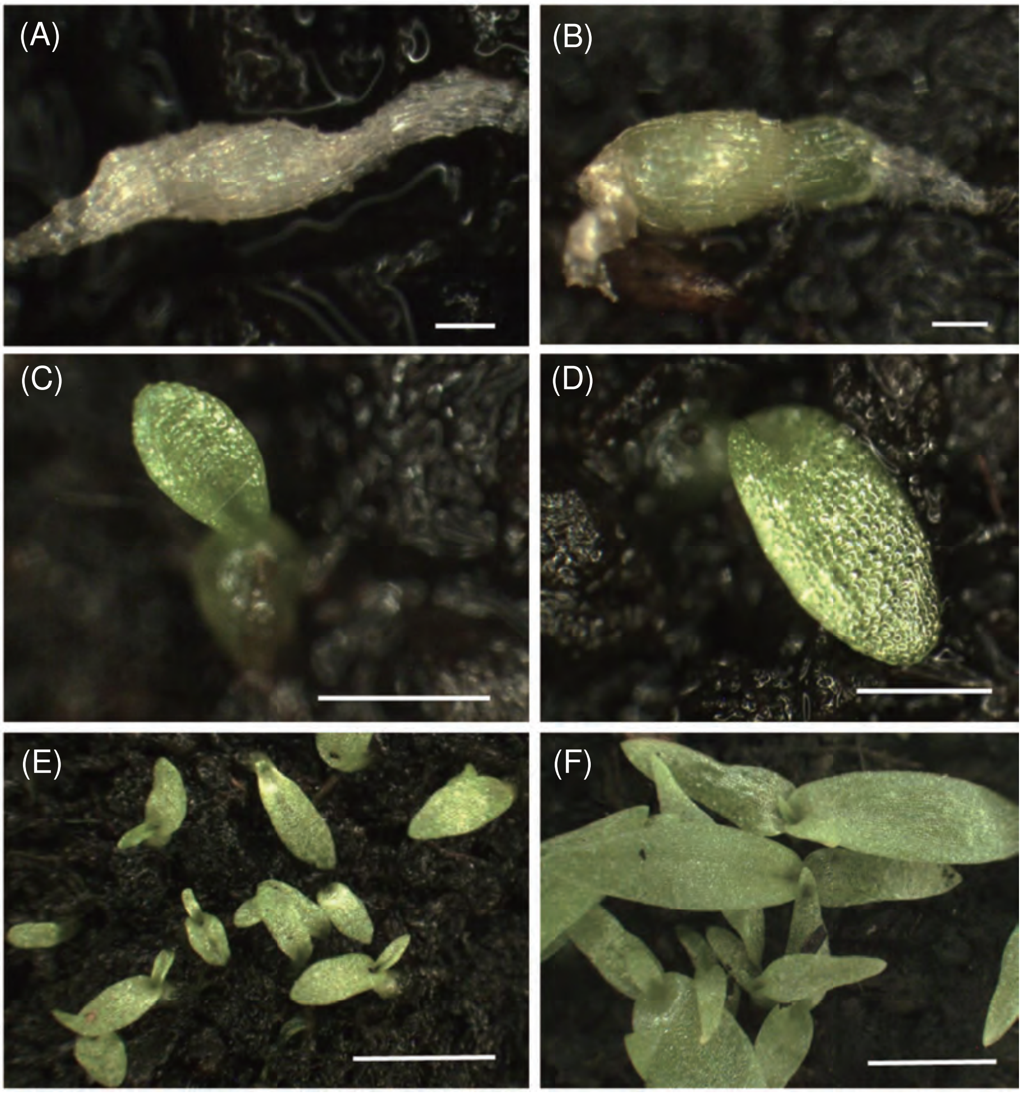

圖 2. 以授粉後 120 d 之台灣白及種子播種於已滅菌處理泥炭土介質，種子發芽與生長之情形。 (A) 播種後 3 d；(B) 播種後 10 d；(C) 播種後 17 d；(D) 播種後 31 d；(E) 播種後 60 d；(F) 播種後 120 d。(A–B) bar = 0.1 mm；(C–D) bar =1 mm；(E–F) bar = 3 mm。

Fig. 2. Germination and seedlings growth of *Bletilla formosana* (Hayata) Schltr. Seeds were collected from a capsule in 120 days after pollinating and were sown in an autoclaved peat moss medium for (A) 3 d; (B) 10 d; (C) 17 d; (D) 31 d; (E) 60 d; and (F) 120 d. (A–B) bar = 0.1 mm; (C–D) bar =1 mm; and (E–F) bar = 3 mm.

種子發芽與生長測試，結果顯示種子可發芽但僅能維持短期之生長。本研究以成熟自然開裂之台灣白及果莢種子，播種於自行混合之炭化稻殼：壤土：細砂 (體積比 = 1 : 1 : 1) 介質中，觀察發現台灣白及種子極易發芽，且可長成完整植株 (資料未顯示)，此種混合介質已有良好發芽與生長效果。因此，本試驗所使用之 2 種混合介質不再添加泥炭土，並減少混合介質的複雜性，以評估是否有更好之發芽與栽培介質。另為排除播種介質中具有菌根菌的可能性，故進行較嚴謹之介質滅菌試驗，並測試不同授粉後天數之種子播種於滅菌之不同介質中，觀察種子發芽及小苗生長之情形，藉以證實台灣白及種子是否具有發芽與自營生長之特性。

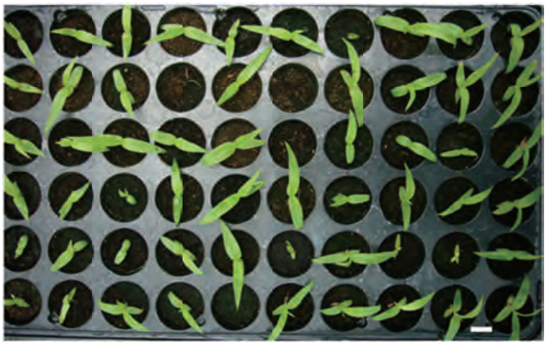

圖 3.　從培養皿移植至含泥炭土生長 60 d 後台灣白及小苗生長情形。Bar = 1 cm。

Fig. 3.　Growth of *Bletilla formosana* (Hayata) Schltr. seedlings after transplanting from *in vivo* cultivation to sterilized peat moss medium for 60 d. Bar = 1 cm.

研究顯示蘭科植物種子成熟度會影響其發芽能力，蝴蝶蘭白花雜交種授粉後 110 d 採收之果莢於無菌播種後 10 d 即可發芽，若延至果莢黃熟甚至開裂才行播種，亦不影響種子發芽能力及原球體發育 (Tu & Lee 1988)。然在萬代蘭屬 (*Vanda*) (Helena Mathews & Rao 1980) 與喜普鞋蘭屬 (*Cypripedium*) (Harvais 1980) 的研究顯示，黃熟期果莢之種子於無菌播種後，種子不發芽或發芽率不佳，甚至影響小苗之生長。本研究取自 5 個不同授粉後天數之台灣白及種子，播種於 5 種完全滅菌之單一或混合介質中進行發芽 (表 2)，顯示授粉後 60 d 之種子即具有發芽能力，播種於泥炭土後 16 d 即可發芽。隨著種子成熟度增加，於授粉後 120 d (果莢黃熟) 及 165 d (果莢開裂後 15 d) 之種子分別播種於泥炭土、砂：壤土：炭化稻殼：珍珠石及砂：炭化稻殼：珍珠石：蛭石混合介質，可縮短發芽天數於 9–10 d。此結果與 Tsao *et al.* (2011) 取授粉後 120 d 黃熟台灣白及果莢之種子，以無菌播種方式於播種後 8 d 可見種子發芽之時間相近，但與 Lin *et al.* (1994) 的研究指出果莢黃熟尚未開裂之種子無法發芽之結果不同，推測原因可能與材料來源、播種處理及培養方式不同而有所差異。除授粉後 90 d 之種子在未滅菌之炭化稻殼介質、砂：壤土：炭化稻殼：珍珠石及砂：炭化稻殼：珍珠石：蛭石混合介質中發芽天數較滅菌介質短之外，大多數之介質滅菌與否並不影響不同授粉後天數種子的發芽時間 (表 2)。惟對播種後之發芽率及存活率的影響，則極為顯著。授粉後 90 d 種子雖於未滅菌之炭化稻殼介質、砂：壤土：炭化稻殼：珍珠石及砂：炭化稻殼：珍珠石：蛭石混合介質發芽天數較滅菌處理介質為短 (表 2)，但介質滅菌處理後其發芽率及存活率則較未滅菌處理介質高 (表 3)。結果顯示，滅菌處理可去除介質中有害微生物或延緩種子及小苗感染傷害，亦可能與未熟種子狀態有關。

本研究之結果顯示授粉後 60 d 種子雖然已具發芽能力，但小苗存活率低，顯示其種子成熟度仍不足 (未鏡檢)。授粉後 90 d 種子即具有良好之發芽能力，推測此時期之種子應已具備基本之成熟度，且隨著授粉後日數增加，種子成熟度增加，發芽率漸增。但是，此一趨勢在存活率方面表現不一致。在介質方面，除以泥炭土介質與砂：壤土：炭化稻殼：珍珠石混合介質有利於早期發芽，砂：炭化稻殼：珍珠石：蛭石混合介質亦有利於發芽，但在存活率方面，僅砂：壤土：炭化稻殼：珍珠石混合介質 (滅菌) 之表現較為穩定。根據本研究結果顯示，高發芽率不一定相對應有高存活率，發芽率與存活率受種子成熟度、介質種類、滅菌處理因子以及因子間之交感效應等因素影響。授粉後 90 d 之種子，雖然播種於滅菌之砂：壤土：炭化稻殼：珍珠石混合介質發芽率為 95.5%，但其小苗存活率僅 31.3%，而授粉後 120 d 之種子播種於滅菌之砂：壤土：炭化稻殼：珍珠石混合介質之發芽率為 90.0%，小苗存活率可達 73.2%；而授粉後 165 d 之種子播種於滅菌之砂：炭化稻殼：珍珠石：蛭石混合介質雖發芽率為 93.2%，但存活率僅剩 6.6%，主要可能係後端生長過程中受到蟲害及苔癬等試驗干擾因子影響。授粉後 150 d 與 165 d 之種子分別播種於砂：炭化稻殼：珍珠石：蛭石混合介質與砂：壤土：炭化稻殼：珍珠石混合介質，發芽率分別為 76.5–79.6% 以及 82.4–83.2% 相對較高，但存活率則明顯以滅菌介質為佳，仍可達 71.8% 與 77.1%。顯示滅菌介質對小苗生長較為有利 (表 3)，亦需注意在培養過程中遭受外在生物入侵危害小苗生長。

不同介質顯著影響小苗生長與發育，以授

粉後 120 d 之種子播種於 5 種滅菌介質後 120 d，顯示出以富含有機質之泥炭土介質為最佳，可能與台灣白及屬於地生蘭 (terrestrial orchid) 之習性有關。小苗生長與發育較快速，其次為砂：炭化稻殼：珍珠石：蛭石混合介質，最差者為單一介質之水苔及炭化稻殼，小苗生長與發育極為緩慢 (表 4、圖 1)。根據研究顯示，蘭科植物種子在環境適宜時，吸水發芽後其胚膨大突出種皮，此時已有葉綠素形成，但無光合作用能力，於野生狀態下若無菌根菌共生，則無法繼續發育完整植株 (Juang & Lee 1985; Lee 1990)。本研究以授粉後 120 d 之種子播種於滅菌泥炭土介質，觀察台灣白及種子發芽與分化發育之過程，顯示播種後第 10 天之種子胚已膨大轉為綠色，並於第 120 天開始長出第三片葉與根系生長，生長成完整植株 (圖 2)。而將存活之單株小苗移植至已滅菌處理之泥炭土介質可持續正常生長 (圖 3)，證實台灣白及可自行發芽，且不必藉由菌根菌即能自營生長。因此，只要選擇足夠成熟之種子，播種於適合之介質中並給予適量之水分，台灣白及種子無須借助菌根菌或組織培養即可正常發芽並分化成完整植株，與大多數蘭科植物種子之發芽機制有所不同。未來仍需更進一步探討台灣白及種子與其他蘭科植物在構造及胚養分含量與成分組成上是否具有差異性，造成其獨特的種子發芽與自營生長特性。

蘭科植物白及屬之台灣白及在授粉後 60 d 後的種子即有發芽能力，不需經由蘭菌共生與無菌播種之異營生長方式，且可經由種子成熟度與介質種類以及介質滅菌與否等因子來提高其瓶外播種之發芽率。在適量水分管理下即可成功發育成完整植株，顯示台灣白及具有自營發芽及生長之特性。在本研究眾多組合中，以授粉後 120 d 之種子播種於滅菌泥炭土介質中對種子發芽速度、發芽率及小苗生長最為有利，種子發芽率可達 90.0%，小苗存活率達 73.2%。顯示台灣白及種子發芽與生長偏好弱酸性，以及質地細緻鬆軟、透氣與保水之富含有機質之介質。此一自營發芽與成長之模式，雖然在小苗存活率、生長速度與培養效率不及無菌播種模式，但在許多原生地只要選擇適當成熟期種子與介質，即可就地成功培養出完整小苗，不需借助組織培養設備及操作之配合，亦毋須人工接種菌根菌是其優點。此一模式將有助於台灣白及之簡易保存與在地繁殖，更有助原生蘭種之快速復育。

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Effects of Seed Maturity and Growth Media Composition on *In Vivo* Asymbiotic Germination of *Bletilla formosana* (Hayata) Schltr.

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Abstract

Tsao, C. Y., U. C. Chen, T. Y. Wu, and C. N. Hsia. 2021. Effects of seed maturity and growth media composition on *in vivo* asymbiotic germination of *Bletilla formosana* (Hayata) Schltr. J. Taiwan Agric. Res. 70(2):129–139.

This study was conducted to verify the effects of seed maturity, growth media composition and media sterilization on *in vivo* asymbiotic germination and seedlings growth of *Bletilla formosana* (Hayata) Schltr. Seeds collected from pods 60, 90, 120, 150 and 165 d after pollination (DAP) were sown on autoclaved or non-autoclaved media, including sphagnum moss (medium 1), peat moss (medium 2), carbonized rice husk (medium 3), and mixed media of sand : loam : carbonized rice husk : perlite = 1 : 1 : 1 : 1 (v/v) (medium 4) and sand : carbonized rice husk : perlite : vermiculite = 1 : 1 : 1 : 1 (v/v) (medium 5). Results showed that seeds of *B. formosana* were able to germinate and to grow normally on various autoclaved media. Growth media composition, seed maturity and their interactions were found to have statistically significant effect on days needed for germination. However, a less significant effect was found between autoclaved media and seed matured after 120 DAP. In this study, immature 60 DAP seeds were capable to germinate, and the shortest time for germination was observed in 165 DAP seeds. Factors of growth media, autoclave and seed maturity as well as their interactions showed significant effects ($P < 0.01$) on seed germination. It was observed that seeds of 90 DAP had germination rates higher than 80% in the appropriate growth media, and the highest germination rate was found on the autoclaved peat moss (84.1%), followed by the autoclaved medium 4 (81.1%), and the autoclaved medium 5 (72.6%). Although survival rates of seedlings derived from different maturity of seeds sown in various media with or without an autoclave with no consistency, it was found that seeds of 120 DAP sown in appropriate growth media had survival rates higher than 70%. An separated experiment using seeds of 120 DAP was conducted by sowing seeds in various autoclaved or non-autoclaved growth media for evaluation of seedlings growth. Results showed that factor of an autoclave work and types of media as well as their interactions all had statistically significant effects at 0.01 level. It was found that autoclaved peat moss showed the best result on the growth of seedlings. In average, 3.5 leaves and 7.0 mm in leaf span were found per plant. The results confirmed that *B. formosana* had autotrophic feature during seed germination and seedling growth when mature seeds were germinated on appropriate growth medium. It was observed that embryos of seeds were enlarged and turned into green in 10 d after sowing, and the first leaf appeared in 17 d after sowing from protocorms, and a complete plant with roots was obtained in 120 d after sowing. Growth media and maturity of seeds and their interactions were all found had significant effects on days needed for germination. However, a less significant effect by an autoclave was found from the more mature seeds (≥ 120 d). Factors of growth media, autoclaving and seed maturity as well as their interactions showed significant effects ($P < 0.01$) on seed germination and survival of seeding.

Key words: Orchid seed, Asymbiotic germination, Orchid mycorrhiza.

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台灣南部地區番石榴園炭疽病菌 (*Colletotrichum* spp.) 多樣性及對殺菌劑之敏感性

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摘要

蔡蕣隍、陳冠妏、黃健瑞。2021。台灣南部地區番石榴園炭疽病菌 (*Colletotrichum* spp.) 多樣性及對殺菌劑之敏感性。台灣農業研究 70(2):140–156。

番石榴炭疽病是番石榴果實採收期至採收後其中一種重要病害，本研究主要目的在於瞭解目前發生在番石榴園之炭疽病菌族群的主要種類，以及探討這些炭疽病菌族群對核准用來防治炭疽病殺菌劑之敏感性。本試驗於 2019–2020 年間至南部番石榴主要產區採集番石榴炭疽病，採集部位包括無病徵葉片及已產生病斑之病果，將 15 分離株分別接種至「珍珠拔」果實。結果顯示，在有傷口的情況下，大部分菌株皆可造成不等程度的果腐病徵，其中分離自果實菌株的致病力普遍比分離自無病徵葉片強。為了確認所分離之炭疽病菌株種類，將 internal transcribed spacer (ITS)、glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)、β-tubulin 2 (*TUB2*)、chitin synthase 1 (*CHS-1*) 及 calmodulin (*CAL*) 5 個基因序列作定序並建構親緣樹。由建構之番石榴炭疽病族群親緣樹顯示，台灣番石榴炭疽病至少包含 *Colletotrichum siamense*、*Colletotrichum fructicola* 與 *Colletotrichum tropicale* 等 3 種。由藥劑試驗結果顯示，*C. siamense*、*C. fructicola* 對 benzimidazoles 類及 strobilurins 類藥劑普遍呈現不敏感；而 *C. tropicale* 對 benzimidazoles 類藥劑具敏感性，但對 strobilurins 類藥劑呈不敏感，因此 *C. tropicale* 相對於其他兩種炭疽病菌對 benzimidazoles 藥劑具敏感度的差異性。另外，當藥劑濃度 100 mg a.i. L^{-1} 時，克熱淨及鋅錳乃浦可完全抑制 3 種炭疽病菌菌絲生長，當藥劑濃度 1 mg a.i. L^{-1} 時，僅腐絕快得寧及腈硫醌可顯著抑制 3 種炭疽病菌孢子發芽。由以上藥劑試驗結果，可提供農民在田間綜合防治管理炭疽病的參考。

關鍵詞：番石榴、炭疽病菌、族群親緣分析、藥劑敏感性。

前言

番石榴 (*Psidium guajava* L.) 為桃金孃科 (Myrtaceae) 番石榴屬 (*Psidium*) 之熱帶果樹，在台灣各地廣泛種植，近幾年栽培面積多維持在 7,000 多公頃。根據 2018 年農業統計年報 (https://agrstat.coa.gov.tw/sdweb/public/book/Book.aspx)，番石榴栽培面積 7,442 ha，年產量約 176,304 Mg，由栽種面積多寡區分，主要栽培地區依序為高雄市 (2,694 ha)、台南市 (1,467 ha) 及彰化縣 (1,258 ha)。主要栽培品種有「珍珠拔」、「帝王拔」、少數紅肉品種番石榴及「水晶拔」，其餘品種因產量、栽培管理容易度及市場接受度等問題而僅有零星栽培。

在國外，番石榴病害已知被記載的約有 177 種，其中有 167 種病原菌屬於真菌，因此大多數番石榴病害由真菌所引起，其餘的分別是由線蟲、細菌及寄生性藻類危害 (Misra 2004)。在台灣，根據《台灣植物病害名彙》(第五版) 有記錄的病害約 39 種，真菌及類真菌病害約 15 種，線蟲 24 種 (Tzeng *et al*. 2019)。

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台灣地區番石榴病害，由受害部位區分，全株性感染有 *Nalanthamala psidii* 引起的立枯病，可造成植株死亡；根部病害有立枯病及根瘤線蟲 (*Meloidogyne* sp.)；莖幹及枝條有 *Lasiodiplodia theobromae* 引起的莖潰瘍病及 *Phytophthora nicotianae* 造成的疫病；葉部病害有藻斑病、葉斑病及煤煙病；果實病害有黑星病 (病原 *Phyllosticta psidiicola*)、瘡痂病 (病原 *Pestalotiopsis psidii*)、炭疽病 (病原 *Colletotrichum* sp.)、黑腐病 (病原 *L. theobromae*) 及疫病 (病原 *Phytophthora parasitica*)。

番石榴炭疽病根據早期文獻只記載由 *Colletotrichum gloeosporioides* Penz. (1882) 單一菌株所引起 (Penzig 1882)，Von Arx (1957) 及 Sutton (1980) 曾利用炭疽病菌分生孢子之形狀及大小區分 *C. gloeosporioides*，但其分類之依據以現在來看仍不淺顯易懂。在分子生物工具尚未普及前，炭疽病菌屬 (*Colletotrichum*) 的分類通常依據寄主的不同、分生孢子的形狀與大小、附著器的形狀、有無剛毛 (setae)、在培養基的顏色及生長速度等，但真菌培養在不同培養基、不同溫度之生長條件或是重複繼代下會產生變異，於形態鑑定上會有受限的情形。根據國外文獻指出，*C. gloeosporioides* 為一複合種 (species complex)，對於 *C. gloeosporioides* 這個演化支而言，若單純只以 internal transcribed spacer (ITS) 序列去比對仍不足以將之區分開來，以串連的基因序列進行分析，包括 actin (*ACT*)、calmodulin (*CAL*)、chitin synthase 1 (*CHS-1*)、glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) 及 ITS。利用這些基因比對後所構築出的親緣關係樹，顯示該複合種內可再細分成 22 個種及 1 個亞種 (Weir *et al*. 2012)。

在田間炭疽病的防治，目前仍是以化學防治為主。有鑑於近來農民反應使用現行核准藥劑達不到以往之防治效果，且國內外已有多篇報導炭疽病對不同作用機制殺菌劑之抗藥性已在田間被發現，例如台南芒果炭疽病多數菌株對 benzimidazoles 類殺菌劑 [包括貝芬替 (carbendazim)、免賴得 (benomyl) 及腐絕 (thiabendazole)] 產生不同程度藥劑感受性降低的情形 (Lou *et al*. 2010)；而在國外，*Colletotrichum acutatum* 及 *C. gloeosporioides* 引起的蘋果苦腐病 (apple bitter rot) 已對泛醌氧化酶抑制劑 [如亞托敏 (azoxystrobin)、三氟敏 (trifloxystrobin)、克收欣 (kresoxim-methyl)、凡殺同 (famoxadone) 等類] 產生抗藥性 (Chechi *et al*. 2019)。

本研究探討的重點，在於瞭解目前在番石榴植株流行之炭疽病菌種類及其分群，以及測試各個分群之菌株對於現行核准藥劑之敏感性，俾進行抗藥性監測並提供試驗結果作為抗藥性管理之參考。

材料方法

供試菌株分離培養及保存

自屏東縣新園鄉，高雄市燕巢區、大社區、阿蓮區，台南市山上區，分別採集番石榴果實及無病徵之葉片。將具有褐化病徵之果實以滅菌後之解剖刀切取病組織邊緣，病組織以 1% 次氯酸鈉水溶液消毒 3 min，再以 70% 酒精水溶液浸漬 1 min，之後以無菌水漂洗病組織 3 次，移至已滅菌的擦手紙。待病組織水分吸乾後，移至 2% (w/v) 水瓊脂 (water agar; WA) 培養。經於 25℃培養 3 d 後，切取菌絲尖端移至馬鈴薯葡萄糖瓊脂 (potato dextrose agar; PDA) 培養基培養。待產孢後，將孢子塗布於 3% 水瓊脂平板培養基進行單孢分離，將菌株培養於 PDA 斜面，置於室溫備用。

無病徵葉片之分離，以自來水清洗葉片表面，使用 95% 酒精浸漬 1 min，接著 3% 次氯酸鈉水溶液浸泡 5 min，再以 75% 酒精浸泡 30 s，以無菌水清洗 2 次。最後，以滅菌後的解剖刀切取葉片組織 (Dauanla-Meli *et al*. 2013)，移至馬鈴薯葡萄糖瓊脂培養基 25℃培養約 7 d，從葉片長出之炭疽病菌落切取至新的 PDA 平板，待產孢後挑取單孢至 PDA 斜面備用。

炭疽病菌以矽膠粒 (silica gel) 進行長期保存，將矽膠粒盛於可密閉之凍菌小管內，矽膠粒約 7–8 分滿即可。高壓滅菌釜滅菌後，以無菌移植環挑取分生孢子堆之孢子移入 400 μL 7.5% (w/v) 脫脂牛奶，做成真菌孢子濃縮懸浮液。再吸取 350 μL 懸浮液加入盛有矽膠粒之

凍菌小管內，並上下搖動凍菌小管使矽膠粒與懸浮液混合均勻，置於 -20℃保存，7 d 後取少量矽膠粒測試保存之菌有無活力。

炭疽病菌接種及病原性測定

從 PDA 斜面保存管切取一小塊菌絲，置於 PDA 培養基培養約 3 d 後，切取菌絲尖端至 Murashige & Skoog (MS) 平板培養基 (modified Mathur's medium + 0.1% yeast extract + 0.1% Bacto™ peptone + 1% sucrose + 0.25% $MgSO_4 \cdot 7H_2O$ + 0.27% KH_2PO_4 + 2% agar) (Tu 1985) 培養約 1 wk 等待產孢。再以無菌水洗下孢子，利用血球計數器計算孢子濃度，調整濃度為 1×10^6 conidia mL^{-1}，作為果實接種源。供試菌株來自屏東縣新園鄉、高雄市燕巢區、大社區等地區之罹病果所分離之炭疽病菌，以市場購得之新鮮「珍珠拔」果實做接種試驗。先以 1% 次氯酸納消毒果實表面，再以沾濕無菌水之滅菌擦手紙擦拭乾淨，以消毒之解剖刀切出長、寬與深約 1 mm 的傷口，將 10 µL 孢子懸浮液滴在傷口處，同一個果實做 3 重複，並以無菌水作為對照組。置於 25℃黑暗培養 1 wk 後，量取果實接種處的病斑直徑，本試驗共重複 2 次。

各菌株之 DNA 萃取及聚合酶連鎖反應 (polymerase chain reaction; PCR)

試驗於 PDA 平板培養 10 d 的菌株，刮下菌絲後，以 genomic DNA extraction kit (Nautia Gene, Taipei, Taiwan) 抽取 DNA，再利用專一性引子對 (表 1) 進行 PCR 反應擴增 ITS (White *et al*. 1990), *GAPDH* (Guerber *et al*. 2003), *CHS-1* (Carbone & Kohn 1999), β-tubulin 2 (*TUB2*) (O'Donnell & Cigelnik 1997) 及 *CAL* (O'Donnell *et al*. 2000) 等 5 個基因片段，並將 PCR 產物送交定序 (Genomics, New Taipei, Taiwan)。獲得的基因片段序列，以 National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/genbank) 的 Nucleotide Basic Local Alignment Search Tool (BLASTn) 功能進行序列比對。

親緣關係樹之建立

將各菌株的序列，與 *C. gloeosporioides* 種群中的各種菌株的 ITS、*GAPDH*、*TUB2*、*CHS-1* 及 *CAL* 基因片段序列，進行多基因序列分析 (Weir *et al*. 2012)，各參考菌株的序列取自 NCBI 基因庫 (Genbank)。以 MEGA 10 軟體進行序列親緣分析，序列先用 ClustalW 軟體進行排併及適當調整後，再以 maximum likelihood 法進行親緣分析，以瞭解供試菌株與已知 *C. gloeosporioides* 種群中的各菌種的親緣性。

炭疽病菌之形態觀察

將單孢培養之供試菌株繼代至 PDA 平板培養基，於 25℃生長箱 12 h 光照/12 h 黑暗培

表 1. 本研究所使用引子對序列及參考資料來源。
Table 1. The loci and the primers used in this study.

Gene[z]	Primer	Direction	Sequence (5'-3')	Reference
ITS	ITS1	Forward	TCC GTA GGT GAA CCT GCG G	White *et al*. (1990)
	ITS4	Reverse	TCC TCC GCT TAT TGA TAT GC	White *et al*. (1990)
GAPDH	GDF	Forward	GCC GTC AAC GAC CCC TTC ATT GA	Guerber *et al*. (2003)
	GDR	Reverse	GGG TGG AGT CGT ACT TGA GCA TGT	Guerber *et al*. (2003)
TUB2	T1	Forward	AAC ATG CGT GAG ATT GTA AGT	O'Donnell & Cigelnik (1997)
	T2	Reverse	TAG TGA CCC TTG GCC CAGT TG	O'Donnell & Cigelnik (1997)
CAL	CL1	Forward	GAR TWC AAG GAG GCC TTC TC	O'Donnell *et al*. (2000)
	CL2A	Reverse	TTT TTG CAT CAT GAG TTG GAC	O'Donnell *et al*. (2000)
CHS-1	CHS-79F	Forward	TGG GGC AAG GAT GCT TGG AAG AAG	Carbone & Kohn (1999)
	CHS-345R	Reverse	TGG AAG AAC CAT CTG TGA GAG TTG	Carbone & Kohn (1999)

[z] ITS: internal transcribed spacer; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase; *TUB2*: β-tubulin 2; *CAL*: calmodulin; *CHS-1*: chitin synthase 1.

養，10 d 後拍攝記錄培養皿的正反面。分生孢子的觀察，則是取自各供試菌株於 PDA 培養基上分生孢子盤產生的分生孢子，於光學顯微鏡 [Nomarski differential interference contrast (DIC), BX51, Olympus, Tokyo, Japan] 以量測攝像系統 (metric camera) 測量 50 個各供試菌株分生孢子大小。另以玻片培養技術 (slide culture technique) 觀察附著器的形狀，於切塊之 1 cm^2 無菌馬鈴薯葡萄糖瓊脂周圍接種菌絲，並蓋上無菌之蓋玻片。置於室溫培養 14 d 後，取下蓋玻片，置於滴有乳酸的載玻片上觀察附著器。

藥劑對 3 種番石榴炭疽病菌菌絲生長之影響

以目前現有番石榴炭疽病核准藥劑，進行藥劑對菌絲生長抑制的測試。選擇不同作用機制共 13 種核准藥劑 (附錄 1)，分別配製成 3 種濃度：1、10 及 100 mg a.i. L^{-1}，並以未添加藥劑之 PDA 平板培養基作為對照組。將供試菌株 *Colletotrichum siamense* SYCo3、*Colletotrichum fructicola* YCCo5、*Colletotrichum tropicale* ALCo2 從 PDA 斜面移至新鮮的 PDA 培養基，室溫培養約 7 d。以滅菌之打孔器 (孔徑 0.5 cm) 切取菌絲塊，將菌絲塊以面向下的方式移至含不同濃度藥劑之 PDA 平板的中央，置於 25℃生長箱 12 h 光照/12 h 黑暗培養。待對照組長滿培養皿後，測量對照組及藥劑處理組菌落直徑，並以下列公式計算菌絲生長抑制率 (%)：[(對照組平均生長直徑 − 藥劑處理組平均生長直徑)]/對照組平均生長直徑 × 100%。每菌株每藥劑濃度共 4 盤培養皿，本試驗共重複 2 次。

藥劑對 3 種番石榴炭疽病分生孢子發芽之影響

以有效成分 10 mg a.i. L^{-1} 及 1 mg a.i. L^{-1} 藥劑濃度，測試對不同種之番石榴炭疽病發芽影響。以無菌微量吸管吸取 25 μL 含不同藥劑之溶液 (濃度 20 mg a.i. L^{-1})，置於載玻片上，再以無菌微量吸管吸取 25 μL 供試之 *C. siamense* SYCo3、*C. fructicola* YCCo5、*C. tropicale* ALCo2 孢子懸浮液 (1 × 10^5 conidia mL^{-1})，含 0.50 % 葡萄糖，滴於上述含農藥之液滴內，混合均勻，使最終農藥有效成分為 10 mg a.i. L^{-1}，葡萄糖最終濃度 0.25%。另以無菌微量吸管吸取 25 μL 含不同藥劑之溶液 (濃度 2 mg a.i. L^{-1})，置於載玻片上，再以無菌微量吸管吸取 25 μL 供試之 SYCo3、YCCo5、ALCo2 菌株孢子懸浮液 (1 × 10^5 conidia mL^{-1})，含 0.5% 葡萄糖，滴於上述含農藥液滴內，混合均勻，使最終農藥有效成分為 1 mg a.i. L^{-1}，葡萄糖最終濃度 0.25%。供試載玻片置於加有無菌水之玻璃培養皿，將玻璃培養皿置於 25℃生長箱下黑暗培養，經過 18 h 後於顯微鏡逢機計算 100 個孢子之發芽數，發芽率公式如下：(藥劑處理組孢子發芽個數/對照組孢子發芽個數) × 100%。每個處理 1 皿 3 重複，本試驗重複 2 次，共 6 重複。以含 0.25% 葡萄糖之無菌水處理做為對照組。

統計分析

藥劑試驗數據以 SAS 軟體進行單因子變方分析 (analysis of variance; ANOVA)，而後於 5% 顯著水準下以最小顯著性差異 (least significant difference; LSD) 比較同一處理間平均值之差異。

結果

番石榴炭疽病病徵及分離培養結果

2019–2020 年間，自南部地區 (包括屏東縣新園鄉、高雄市燕巢區、大社區、阿蓮區，以及台南市山上區) 將田間疑似炭疽病菌感染的病果及無病徵葉片作例行性採樣及分離培養，從不同品種番石榴分離的炭疽病菌株共有 15 株 (表 2)。炭疽病菌在果實上的病徵，主要是產生不規則或接近圓形褐化病斑 (圖 1A 紅色箭頭，圖 1B 紅圈處)，在高濕的環境下於病斑中心處會產生鮭魚肉色或是深橘色之分生孢子堆。另外，有時候病斑在一小點時會在周圍產生紅色暈環 (圖 1A 黃色箭頭)，病斑中央則明顯凹陷，於凹陷處產生大量分生孢子，當病斑數量多時會融合在一起。至於採集之無明顯病徵葉片 (圖 1C)，經消毒後，葉片組織於 PDA 培養基上產生不同型態之菌落，炭疽病

表 2. 自無病徵葉片及番石榴果實分離之炭疽病各菌株之病原性及毒力。

Table 2. Pathogenicity and virulence of *Colletotrichum* strains isolated from symptomatic guava fruit and symptomless leaves.

Isolate	Location	Part	Symptom	Virulence[z]	Cultivar of guava
YCCo1	Yanchao, Kaohsiung	Fruit	Brown rot	+++	'Zhen-zhu Ba'
YCCo2	Yanchao, Kaohsiung	Fruit	Brown rot	+	'Zhen-zhu Ba'
YCCo3	Yanchao, Kaohsiung	Fruit	Brown rot	++	'Zhen-zhu Ba'
YCCo4	Yanchao, Kaohsiung	Fruit	Brown rot	+++	'Zhen-zhu Ba'
YCCo5	Yanchao, Kaohsiung	Leaf	Symptomless	++	Guava 'TNG1'
YCCo6	Yanchao, Kaohsiung	Leaf	Symptomless	++	Guava 'TNG1'
SYCo1	Sinyuan, Pingtung	Fruit	Brown rot	+++	'Shui-jing Ba'
SYCo2	Sinyuan, Pingtung	Fruit	Brown rot	+++	'Shui-jing Ba'
SYCo3	Sinyuan, Pingtung	Fruit	Brown rot	+++	'Shui-jing Ba'
SYCo4	Sinyuan, Pingtung	Fruit	Brown rot	++	'Shui-jing Ba'
ALCo1	Alien, Kaohsiung	Pedicle	Symptomless	++	'Hong Zuan'
ALCo2	Alien, Kaohsiung	Fruit	Brown rot	+++	'Hong Zuan'
ALCo3	Alien, Kaohsiung	Fruit	Brown rot	++	'Hong Zuan'
SSCo1	Shanshang, Tainan	Leaf	Symptomless	+	'Hong Zuan'
DSCo1	Dashe, Kaohsiung	Leaf	Symptomless	+	'Bai Ba'

[z] The virulence of each isolate is distinguished by the diameter of the lesions. Disease scale bar is as follow: -, 0 mm; +, < 15mm; ++, 15–20 mm; and +++, > 20 mm.

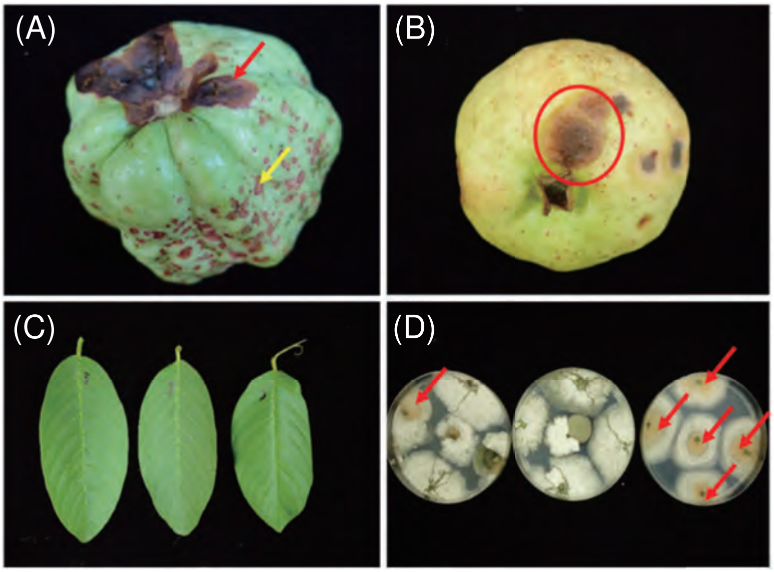

圖 1. (A)「水晶拔」果實由 *Colletotrichum* sp. 所引起之炭疽病病徵，果頂為不規則之褐色病斑，病斑中央呈黑褐色凹陷 (紅色箭頭)，果身之小斑點周圍有紅色暈環，中央呈褐色凹陷狀 (黃色箭頭)；(B)「紅鑽」品種果實之炭疽病病徵，呈圓形至不規則形之病斑 (紅圈處)；(C)「珍珠拔」之無病徵葉片；(D) 以嚴苛消毒方法分離培養之炭疽病菌落 (紅色箭頭)。

Fig. 1. (A) Symptoms of anthracnose caused by *Colletotrichum* sp. with irregular brown spots and dark brown sunken lesion at the center of cv. 'Shui-jing Ba' (red arrowed). Sometimes there are small brown sunken spots with red haloes on pericarp. (yellow arrowed). (B) Rounded to irregular lesion (red circle) of anthracnose on the fruit of cv. 'Hong Zuan'. (C) Symptomless leaves of cv. 'Zhen-chu Ba'. (D) Colonies of *Colletotrichum* sp. (red arrowed) isolated from symptomless leaves after strict disinfection.

菌之菌落通常會在中央產生橘色之分生孢子堆(圖 1D 紅色箭頭)。若無孢子，菌落正面通常呈白色毛絨狀，背面會產生淡灰色之色素，但仍有誤判可能，須進一步將之繼代培養至 MS 產孢培養基鏡檢後確認。

炭疽病菌接種及病原性確認

將各地區感染不同番石榴品種及不同組織獲得之炭疽病菌株進行收集保存，為確認其病原性，以「珍珠拔」作為接種供試品種，其結果如表 2 所示。自具病徵果實或無病徵葉片之菌株，皆可造成番石榴產生不同程度的褐色果實腐爛病徵，切開接種處，同樣可觀察到褐色至黑色之果腐病斑，表示這些菌株皆對番石榴具有病原性。經再一次分離後，獲得同樣形態之菌株，完成科霍氏法則。另外，分離自果實之菌株普遍毒力 (virulence) 較葉片菌株強 (表 2)，且造成感染的條件需要有傷口，若無傷口則無法進一步感染果實 (Tsai，未發表資料)。

番石榴炭疽病菌親緣分析

將15株番石榴炭疽病菌的ITS序列以NCBI GenBank 的 BLASTn 比對，這 15 株菌株與 *C. gloeosporioides* 複合種內菌株之序列具有高於 99% 的相同性，表示這 15 株番石榴炭疽病菌菌株歸屬於 *C. gloeosporioides* 複合種。為了精確鑑定番石榴炭疽病菌，故使用 ITS、*GAPDH*、*TUB2*、*CHS-1* 及 *CAL* 基因片段序列 (Weir *et al*. 2012) 進行多基因序列分析 (附錄 2)。親緣關係圖顯示，15 個菌株中有 10 個菌株與 *C. siamense* ICMP 18578 歸於同一演化分支，3 個菌株 (YCCo5, DSCo1, YCCo2) 與 *C. fructicola* LF130 歸於同一演化分支，1 個菌株 (SSCo1) 與 *C. fructicola* ITCC 6270 歸於同一演化分支，另 1 菌株 (ALCo2) 則與 *C. tropicale* ICMP18653 為同一群。由此知，造成台灣番石榴炭疽病至少包含 *C. gloeosporioides* 複合種中 *C. siamense*、*C. fructicola* 與 *C. tropicale* 等 3 種炭疽病菌 (圖 2)。

三種供試菌株菌落、分生孢子及附著器型態

供試之 *C. siamense* SYCo3、*C. tropicale* ALCo2、*C. fructicola* YCCo5 於 25℃培養於馬鈴薯葡萄糖瓊脂培養基 10 d 後，於正面的菌落中央普遍呈灰白色，菌絲棉質且稠密，邊緣則呈白色。中央部位偶爾可見已泌出鮭魚肉色分生孢子之分生孢子盤，背面也呈灰白色，偶爾可見一些灰黑色色素的沉澱 (圖 3)。3 種炭疽病菌之分生孢子均呈透明、無隔、短圓柱形且外壁平滑 (圖 4A–4C)，*C. siamense* SYCo3 分生孢子長寬約為 11.3–14.4–16.5 μm × 4.4–5.1–5.9 μm，*C. tropicale* ALCo2 分生孢子長寬約為 12.1–15.9–18.8 μm × 4.5–5.2–5.9 μm、*C. fructicola* YCCo5 分生孢子長寬約為 12.8–15.1–19.2 μm × 3.7–4.9–5.7 μm。另外，*C. siamense* SYCo3 及 *C. fructicola* YCCo5 附著器形狀多變化，多呈不規則形凸起，少數邊緣淺裂。*C. tropicale* ALCo2 則多為橄欖形，但也有不規則形 (圖 4D–4F)。

番石榴炭疽病核准藥劑對供試菌株菌絲生長的影響

核准藥劑對不同種炭疽病菌菌絲生長的影響，結果如表 3 所示。當藥劑濃度 1 mg a.i. L^{-1} 時，僅扶吉胺可分別抑制 *C. siamense* SYCo3、*C. tropicale* ALCo2 及 *C. fructicola* YCCo5 的菌絲生長達 68.8、90.6 及 81.1%。當藥劑有效成分濃度為 10 mg a.i. L^{-1} 時，僅克熱淨及扶吉胺可使 3 種炭疽病菌菌絲生長抑制率同時達到 80% 以上。以克熱淨為例，3 株菌 *C. siamense* SYCo3、*C. tropicale* ALCo2 及 *C. fructicola* YCCo5 的菌絲生長抑制率分別為 100.0、87.6 及 99.4%。當藥劑有效成分濃度達到 100 mg a.i. L^{-1} 時，僅克熱淨及鋅錳乃浦對 3 種炭疽病菌的菌絲生長抑制率均達到 100%，而甲基多保淨 (thiophanate-methyl) 則僅對 *C. tropicale* ALCo2 的菌絲生長抑制率達到 100%。另外，2 種炭疽病菌則對甲基多保淨不敏感，而賽普護汰寧、得克利及扶吉胺均可抑制 3 種炭疽病菌菌絲生長達 80% 以上。亞托敏、克收欣、三氟派瑞 (trifloxystrobin + fluopyram) 等 strobilurins 類藥劑普遍對 3 種炭疽病菌無明顯之菌絲生長抑制效果，菌絲生長抑制率均在 25% 以下。

番石榴炭疽病核准藥劑對供試菌株孢子發芽的影響

藥劑濃度為 10 mg a.i. L^{-1} 時，賽普護汰寧、扶吉胺、得克利、免得爛、腐絕快得寧及腈硫

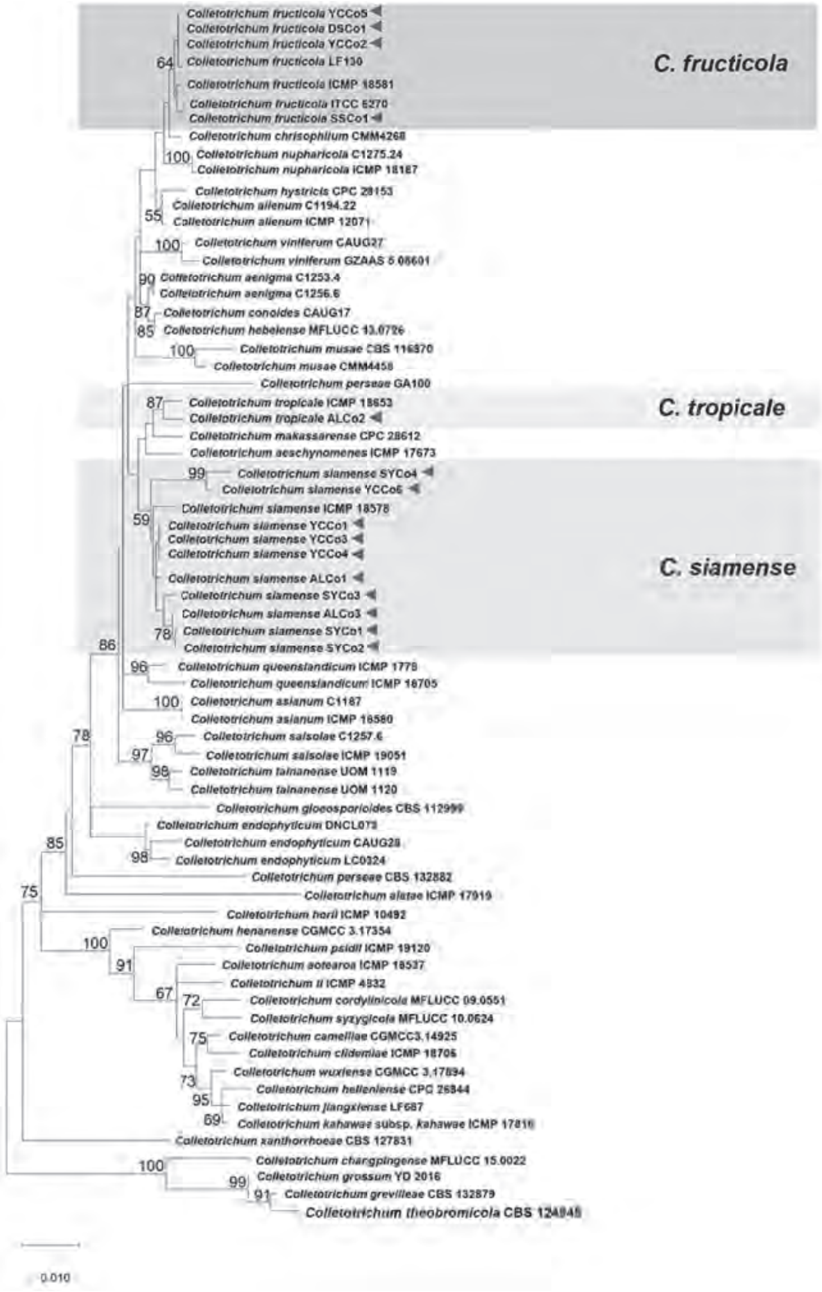

圖 2. 以多基因串連序列 (ITS, *GAPDH*, *TUB2*, *CHS-1* 及 *CAL*) 藉由最大似然法分析本研究之 15 菌株與 *Colletotrichum gloeosporioides* 複合種之 54 菌株比對所建構之親緣樹。15 菌株有 10 菌株歸類在 *Colletotrichum siamense*，4 菌株歸類在 *Colletotrichum fructicola*，1 菌株在 *Colletotrichum tropicale* (箭頭所示)。節點上的數值代表分析 1,000 次重複之 bootstrap value，比例尺代表預估之核苷酸置換率。

Fig. 2. Phylogenetic tree of 15 *Colletotrichum* isolates collected in this study (arrowed) and 54 isolates within *C. gloeosporioides* complex. The tree was built using concatenated sequences of the internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β-tubulin 2 (*TUB2*), chitin synthase 1 (*CHS-1*) and calmodulin (*CAL*) genes and analyzed by maximum likelihood method. Of the 15 isolates, 10 isolates are classified as *C. siamense*, 4 isolates are classified as *C. fructicola* while 1 isolate is classified as *C. tropicale*. Numbers above the nodes represent the bootstrap values (1,000 replicates). Scale bar indicates the number of expected changes per nucleotide.

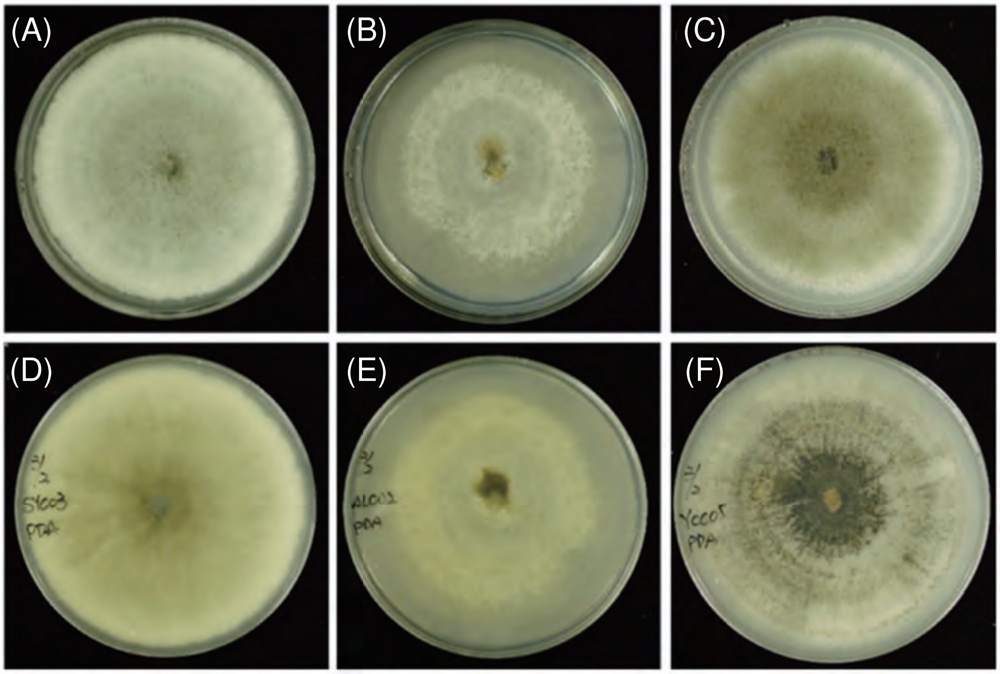

圖 3. 供試之 (A、D) *Colletotrichum siamense* isolate SYCo3；(B、E) *Colletotrichum tropicale* ALCo2；(C、F) *Colletotrichum fructicola* isolate YCCo5 培養於馬鈴薯葡萄糖瓊脂培養基 10 d (25℃) 後之菌落 (A–C，正面；D–F，背面)。

Fig. 3. Colonies of (A, D) *C. siamense* isolate SYCo3, (B, E) *C. tropicale* ALCo2 and (C, F) *C. fructicola* isolate YCCo5 cultivated on potato dextrose agar at 25℃ for 10 d (A–C, above side; D–F, below side).

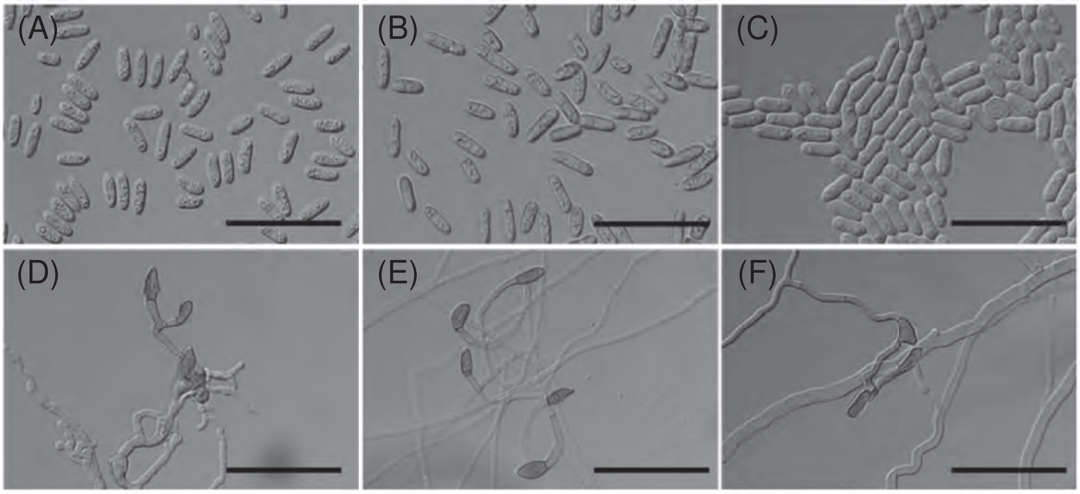

圖 4. 供試之 (A、D) *Colletotrichum siamense* isolate SYCo3；(B、E) *Colletotrichum tropicale* ALCo2；(C、F) *Colletotrichum fructicola* isolate YCCo5 菌株分生孢子及附著器型態觀察 (A–C，分生孢子；D–F，附著器；比例尺 = 50 μm)。

Fig. 4. Conidia and appresoria of (A, D) *C. siamense* isolate SYCo3, (B, E) *C. tropicale* ALCo2 and (C, F) *C. fructicola* isolate YCCo5 (A–C, conidia; D–F, appresoria; scale bar = 50 μm).

表 3. 番石榴炭疽病核准藥劑對不同炭疽菌株菌絲生長的影響。

Table 3. Effect of approved fungicides for guava anthracnose on mycelial growth of different *Colletotrichum* sp. isolates.

	Inhibition (%)[z]								
	1 mg a.i. L^{-1}			10 mg a.i. L^{-1}			100 mg a.i. L^{-1}		
Fungicide	SYCo3[y]	ALCo2[x]	YCCo5[w]	SYCo3	ALCo2	YCCo5	SYCo3	ALCo2	YCCo5
Cyprodinil + fludioxonil	38.2 ± 1.2 c[v]	48.3 ± 1.2 c	18.0 ± 1.4 c	78.8 ± 0.5 c	49.3 ± 2.7 e	65.1 ± 1.9 d	93.0 ± 0.2 b	83.9 ± 0.3 e	97.1 ± 1.4 b
Kresoxim-methyl	0.0 ± 0.0 e	15.1 ± 0.2 d	0.0 ± 0.0 f	0.0 ± 0.0 g	15.1 ± 0.4 h	0.0 ± 0.0 h	0.0 ± 0.0 f	15.8 ± 1.0 g	0.0 ± 0.0 g
Iminoctadine	61.3 ± 1.4 b	0.0 ± 0.0 e	0.0 ± 0.0 f	100.0 ± 0.0 a	87.6 ± 1.3 ab	99.4 ± 0.6 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Azoxystrobin	0.0 ± 0.0 e	0.6 ± 0.6 e	0.0 ± 0.0 f	0.0 ± 0.0 g	2.0 ± 1.0 i	0.6 ± 0.4 h	0.0 ± 0.0 f	23.3 ± 1.1 f	0.0 ± 0.0 g
Fluazinam	68.8 ± 1.3 a	90.6 ± 0.9 a	81.1 ± 1.1 a	82.4 ± 0.3 b	90.1 ± 0.4 a	82.6 ± 2.6 b	89.5 ± 0.5 c	93.3 ± 0.6 b	89.5 ± 2.6 c
Tebuconazole	25.8 ± 1.3 d	50.0 ± 1.1 c	29.0 ± 0.7 b	72.0 ± 0.7 d	69.9 ± 0.4 c	69.5 ± 0.5 c	87.5 ± 1.5 c	91.9 ± 0.5 b	94.9 ± 1.2 b
Thiophanate-methyl	0.0 ± 0.0 e	61.9 ± 0.7 b	0.0 ± 0.0 f	0.0 ± 0.0 g	86.1 ± 1.3 b	0.0 ± 0.0 h	0.0 ± 0.0 f	100.0 ± 0.0 a	0.0 ± 0.0 g
Metiram	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 f	0.5 ± 0.5 g	0.0 ± 0.0 i	0.0 ± 0.0 h	0.0 ± 0.0 f	0.0 ± 0.0 i	0.0 ± 0.0 g
Iprodione	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 f	56.5 ± 0.8 e	62.8 ± 0.7 d	56.1 ± 1.6 e	77.5 ± 0.2 d	83.4 ± 0.7 e	84.3 ± 0.4 d
Thiabendazole + oxine copper	0.0 ± 0.0 e	0.0 ± 0.0 e	14.0 ± 1.0 d	23.0 ± 1.0 f	87.8 ± 1.3 ab	46.9 ± 2.2 f	50.8 ± 2.2 e	89.3 ± 1.7 c	58.6 ± 0.9 e
Dithianon	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 f	0.0 ± 0.0 g	0.0 ± 0.0 i	0.0 ± 0.0 h	1.8 ± 0.7 f	8.0 ± 1.0 h	0.9 ± 0.5 g
Mancozeb	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 f	24.5 ± 1.6 f	27.6 ± 0.7 f	0.0 ± 0.0 h	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Trifloxystrobin + fluopyram	0.0 ± 0.0 e	14.4 ± 1.7 d	3.6 ± 0.4 e	0.0 ± 0.0 g	22.1 ± 0.6 g	8.9 ± 0.4 g	0.0 ± 0.0 f	24.5 ± 1.0 f	6.3 ± 1.0 f

[z] Inhibition rate = [(Mean of mycelial growth of the control – Mean of mycelial growth of the treatment)]/mean of mycelial growth of the control × 100%.

[y] *Colletotrichum siamense* isolate.

[x] *Colletotrichum tropicale* isolate.

[w] *Colletotrichum fructicola* isolate.

[v] Mean ± standard error (n = 8). Means within a column followed by the same letters are not significantly different at P = 0.05 based on least significant difference (LSD) test.

醌處理組皆可有效抑制 *C. siamense* SYCo3、*C. tropicale* ALCo2 及 *C. fructicola* YCCo53 種炭疽病菌株孢子發芽 (表 4)；當藥劑濃度降低至 1 mg a.i. L^{-1} 時，僅扶吉胺、腐絕快得寧及腈硫醌處理組可抑制 SYCo3、ALCo2 及 YCCo5 三種炭疽病菌株孢子發芽率至 1% 以下。

討論

Colletotrichum spp.引起的植物炭疽病 (anthracnose) 為造成果實採收前及採收後重要的病害，係果實生產及外銷的限制因子之一 (Hindorf 2000; Hyde *et al*. 2009; Ismail *et al*. 2015)。本屬真菌寄主範圍廣泛，可感染多種熱帶及溫帶果樹，例如柑橘、香蕉、火龍果、芒果、木瓜、草莓……等 (Phoulivong *et al*. 2010)，番石榴也是炭疽病菌寄主之一 (Hindorf 2000)。本研究自南部地區收集不同品種番石榴果實上獲得的炭疽病菌，所產生的病徵多為濕潤棕褐色腐爛圓形至不規則狀病斑，果表在高濕環境下易產生橘紅色分生孢子堆。由於炭疽病菌亦為陸生熱帶植物內常見的內生真菌，據估計有大約 2,200 種植物有紀錄到此菌 (Rodrigues & Petrini 1997; Vieira *et al*. 2014; Guarnaccia *et al*. 2019)，內生真菌可在活植物組織內蔓延生長而不會造成病徵 (Petrini 1991)，故本研究另外針對無病徵葉片進行採樣，以嚴謹之消毒方法進行分離。結果顯示，葉片內有多種真菌甚至細菌存在，包括 *Phomopsis* sp.、*Phyllosticta* sp.、*Colletotrichum* sp.、*Alternaria* sp. ……等，其中以擬莖點黴屬 (*Phomopsis* sp.) 出現頻率最高 (Tsai，未發表資料)。這些真菌皆為常見之內生真菌，多具潛伏感染特性。Prusky (1996) 研究指出，潛伏感染多發生在營養生長期、花期及果實成熟前期，但明顯的病徵表現則發生在採收後期。為了確定從葉片及果實分離之炭疽病菌株是否有病原性，以果實進行接種測試。若果實表面有傷口的狀況下，所有分離自果實或無病徵葉片炭疽病菌株皆有病原性 (pathogenicity)，但無病徵葉片菌株的毒力 (virulence) 普遍低於具病徵果實的菌株 (表 2)。雖然如此，此結果仍

表 4. 番石榴炭疽病核准藥劑對不同炭疽菌株孢子發芽的影響。

Table 4. Effect of approved fungicides for guava anthracnose on spore germination of different *Colletotrichum* sp. isolates.

	Germination rate (%)[z]					
	1 mg a.i. L^{-1}			10 mg a.i. L^{-1}		
Fungicide	SYCo3	ALCo2	YCCo5	SYCo3	ALCo2	YCCo5
Cyprodinil + fludioxonil	0.0 ± 0.0 e[y]	12.4 ± 1.9 e	13.0 ± 2.2 e	0.0 ± 0.0 f	0.0 ± 0.0 c	0.0 ± 0.0 d
Kresoxim-methyl	70.1 ± 3.2 c	38.4 ± 2.6 cd	86.4 ± 1.8 b	25.7 ± 1.8 d	28.1 ± 9.6 a	72.9 ± 6.1 b
Iminoctadine	81.2 ± 3.7 b	39.4 ± 5.8 bcd	76.9 ± 1.6 c	48.4. ± 2.0 b	27.2 ± 6.4 a	75.8 ± 3.6 b
Azoxystrobin	62.2 ± 4.5 c	41.6 ± 5.1 bc	95.8 ± 1.1 a	22.6 ± 3.0 d	36.5 ± 2.6 a	85.4 ± 5.2 a
Fluazinam	0.0 ± 0.0 e	0.2 ± 0.2 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 c	0.0 ± 0.0 d
Tebuconazole	92.9 ± 1.1 a	88.0 ± 3.0 a	77.2 ± 3.3 cd	0.0 ± 0.0 f	0.0 ± 0.0 c	0.0 ± 0.0 d
Thiophanate-methyl	93.5 ± 1.9 a	39.1 ± 3.7 bcd	78.2 ± 2.1 cd	83.7 ± 2.4 a	30.7 ± 4.3 a	62.9 ± 5.7 c
Metiram	91.5 ± 2.0 a	0.8 ± 0.5 f	79.7 ± 3.3 c	0.0 ± 0.0 f	0.0 ± 0.0 c	0.0 ± 0.0 d
Iprodione	38.4 ± 4.9 d	32.5 ± 1.7 d	73.5 ± 1.4 cd	12.5 ± 2.1 e	28.9 ± 3.3 a	70.8 ± 3.1 bc
Thiabendazole + oxine copper	0.0 ± 0.0 e	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 c	0.0 ± 0.0 d
Dithianon	0.0 ± 0.0 e	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 c	0.0 ± 0.0 d
Mancozeb	69.2 ± 6.2 c	0.0 ± 0.0 f	72.1 ± 3.9 d	0.0 ± 0.0 f	0.0 ± 0.0 c	4.1 ± 2.8 d
Trifloxystrobin + fluopyram	67.7 ± 1.4 c	44.2 ± 6.0 b	92.7 ± 1.8 ab	36.6 ± 2.8 c	16.4 ± 1.8 b	89.9 ± 3.1 a

[z] Spore germination rate = (No. of germinated spores in 0.25% glucose solution with fungicide/No. of germinated spores in 0.25% glucose solution without fungicide) × 100%.

[y] Mean ± standard error (n = 6). Means within a column followed by the same letters are not significantly different at P = 0.05 based on least significant difference (LSD) test.

無法說明內生性炭疽病菌實際的致病力 (virulence potential)，因為本研究是在人工環境以人工接種的方式評估，實際上在自然環境下的致病力仍未知。狹義上來說，內生真菌是指該真菌纏聚在植物體內而不會表現肉眼可見的病徵 (Petrini 1991)，但炭疽菌屬可能是具有內生生活史的伺機性病原菌 (Wilson 1995)。

造成果腐的炭疽病菌以往均認為是由單一菌株 *C. gloeosporioides* Penz. 所引起，但越來越多研究顯示，該菌其實是一個複合種 (Phoulivong *et al*. 2010; Weir *et al*. 2012)。以 ITS 基因片段分析 15 菌株，其基因序列超過 99% 相似性，為更準確區分複合種內的各種炭疽病菌，使用 ITS、*GAPDH*、*TUB2*、*CHS-1* 及 *CAL* 基因片段序列進行多基因序列分析 (Weir *et al*. 2012)。結果顯示，目前台灣番石榴炭疽病在 *C. gloeosporioides* 複合種，包含 *C. siamense*、*C. fructicola* 與 *C. tropicale* 等至少 3 種炭疽病菌，其中 *C. siamense* 占大多數，10 個菌株歸於此演化支，另有 4 個菌株屬於 *C. fructicola*，*C. tropicale* 則僅 1 個菌株 (圖 2)。*C. siamense* 最初是從泰國的咖啡果實炭疽病分離獲得 (Prihastuti *et al*. 2009)，原本有報導指出 *C. siamense* 為一複合種 (Sharma *et al*. 2015)，但之後有學者否定這說法，並以譜系一致的系統發育學種識別法 (Genealogical Concordance Phylogenetic Species Recognition; GCPSR) 確認該種非複合種。因此，後續雖有許多炭疽病新種被發表，例如 *Colletotrichum communis*、*Colletotrichum dianesei*、*Colletotrichum endomangiferae*、*Colletotrichum hymenocallidis*、*Colletotrichum jasmini-sambac*、*Colletotrichum melanocaulon* 及 *Colletotrichum murrayae*，但被證實這些都是 *C. siamense* 的同物異名 (Liu *et al*. 2016)。該菌寄主極為廣泛，許多熱帶、溫帶果樹及蔬菜等園藝作物均可被感染，於芒果、釋迦、木瓜、無花果、辣椒、茶樹、柿子等作物均有被報導 (Udayanga *et al*. 2013; Hassan *et al*. 2018)。與 *C. siamense* 一樣，*C. fructicola* 也是從咖啡果實分離獲得的新種炭疽病菌 (Prihastuti *et al*. 2009)，該菌寄主廣泛，包括草莓、芒果、茶樹等等，並曾被記載為植物內生真菌 (Jayawardena *et al*. 2016)。*C. tropicale* 第一次被報導是在熱帶美洲的刺果番荔枝 (*Annona muricata*) 等熱帶植物被發現，並在可可樹 (*Theobroma cacao*) 葉肉組織內分離到該菌 (Rojas *et al*. 2010)。這 3 種炭疽病菌均隸屬於 *C. gloeosporioides* 複合種，在研究比較透徹的芒果炭疽病，國外正式被記錄的有 20 種 (Fuentes-Aragón *et al*. 2020)，而國內至少有 5 種芒果炭疽病菌被明確記錄，種類包括 *Colletotrichum asianum*、*C. siamense*、*C. fructicola*、*C. tropicale*、*Colletotrichum scovillei*，其中 *C. asianum* 被分離的頻率最高 (Wu *et al*. 2020)。至於番石榴炭疽病，以目前可搜尋到的國內外文獻報告，均沒有明確研究番石榴炭疽病族群多樣性的研究報告，許多都是零星記錄。本篇研究是國內第一次初步記錄南部地區番石榴炭疽病族群種類的報告。

番石榴炭疽病的防治目前仍著重於化學防治，但長期使用慣行農法的農民漸漸反應部分藥劑不如以往有效，為瞭解這個現象的原因，本研究收集與觀察田間番石榴炭疽病族群對目前核准藥劑的敏感性。以建構好之炭疽病族群親緣樹為依據，將 3 種炭疽病菌中各挑選一株毒力較強的菌株作藥劑敏感測試。結果顯示，藥劑濃度 100 mg a.i. L^{-1} 時，克熱淨及鋅錳乃浦可完全抑制 3 種炭疽病菌菌絲生長；藥劑濃度 10 mg a.i. L^{-1} 時，賽浦護汰寧、扶吉胺、得克利、免得爛、腐絕快得寧及腈硫醌可完全抑制 3 種炭疽病菌孢子發芽；當藥劑濃度 1 mg a.i. L^{-1} 時，則只有腐絕快得寧及腈硫醌可完全抑制 3 種炭疽病菌孢子發芽。惟以上藥劑，對 3 種炭疽病菌的抑制效果均無明顯差異性。

另一方面，3 種炭疽病菌對部分核准藥劑的敏感度存在差異性，例如在甲基多保淨藥劑濃度 100 mg a.i. L^{-1} 時，對於 *C. tropicale* ALCo2 可 100% 抑制其菌絲生長，而孢子發芽抑制試驗在藥劑濃度 10 mg a.i. L^{-1} 時，發芽率 30.7%；而 *C. siamense* SYCo3 及 *C. fructicola* YCCo5 在甲基多保淨濃度 100 mg a.i. L^{-1} 時，菌絲生長抑制率均為 0%，藥劑濃度為 10 mg a.i. L^{-1} 時孢子發芽率則分別為 83.7% 及 62.9%，顯示相對於 *C. siamense* 及 *C. fructicola*，*C. tropicale* 對甲基多保淨呈現不同的敏感性或耐受程度。

甲基多保淨與貝芬替、免賴得及腐絕快得寧皆為 methyl benzimidazole carbamate (MBC) 殺菌劑，作用機制皆為抑制有絲分裂過程 β-微管素 (β-tubulin) 的組合。已知抗藥性與 β-tubulin 2 基因上兩處點突變 (E198A/G/K及F200Y) 有關 (Young *et al*. 2010)，炭疽病菌株對此類藥劑呈現抗藥性的現象國外也有研究證實 (Ramdial *et al*. 2016)，且 benzimidazole 類藥劑相較於其他種藥劑有更高的抗藥性風險 (Cao *et al*. 2017)。在墨西哥，有報導指出造成芒果炭疽病的其中 1 種炭疽病菌 (*C. siamense*) 對 benzimidazole 類藥劑有高度抗藥性，即使甲基多保淨藥劑濃度達到 1,000 μg mL^{-1}，該菌仍可於平板培養生長 (Fuentes-Aragón *et al*. 2020)。除此之外，本研究亦發現 *C. fructicola* 在藥劑濃度 100 mg a.i. L^{-1} 時，對甲基多保淨不敏感，在其他報告中，自草莓及山藥分離的 *C. fructicola* 也有同樣的結論 (Han *et al*. 2018)。顯示部分炭疽病菌種類可能已對 benzimidazoles 類藥劑產生不同程度的抗藥性，在抗藥性管理策略的擬定上需要注意。

本研究於生體外 (*in vitro*) 所測試的 strobilurins 類藥劑，包括單劑之克收欣、亞托敏及混合劑之三氟派瑞，結果顯示菌絲生長抑制率對 3 種炭疽病菌均不佳，皆在 25% 以下，且這些藥劑皆無法完全抑制供試菌株孢子發芽。探究其可能原因，國外有研究顯示在真菌粒線體細胞膜上的替代氧化酵素 (alternative oxidase; AOX) 可使真菌在生體外避開 strobilurins 類藥劑產生的致毒作用，造成在生體外測試時產生藥效不佳的現象。另一方面，真菌在生體內 (*in vivo*) 感染植體時，推測 AOX 會被植物的次級代謝物抑制，進而使藥劑發揮藥效 (Wood & Hollomon 2003; Kaneko & Ishii 2009)。

本研究所收集的炭疽病菌株大部分皆來自施行慣行農法的果園，據筆者詢問於燕巢地區施行慣行農法的農民表示，亞托敏、甲基多保淨及腐絕快得寧皆是常用的藥劑。由於田間環境因子複雜，且每一位農民的田間管理 (施藥方法、施藥種類、使用之肥料、水分管理) 皆不同，要真正判斷炭疽病菌株是否對 strobilurins 類藥劑產生抗藥性，仍需於後續研究實際在田間施用 strobilurins 類藥劑，才能真正測試真菌對該類藥劑的敏感性。

綜上所述，番石榴炭疽病菌不只存在於肉眼可見病徵的果實，尚還存活於無病徵之健康葉片，以果實接種兩種不同分離部位的炭疽病菌證實均有病原性，但各菌株之間存在不同程度的致病力。因此，田間管理的首務仍須著重清園，將落葉及落果撿拾集中清理，以降低田間殘存的病原菌密度。另外，由建構之番石榴炭疽病族群親緣樹顯示，可知台灣番石榴炭疽病至少包含 *C. siamense*、*C. fructicola* 與 *C. tropicale* 等 3 種。由藥劑試驗結果顯示，*C. siamense*、*C. fructicola* 對 benzimidazoles 類及 strobilurins 類藥劑普遍呈現不敏感，而 *C. tropicale* 對 benzimidazoles 類藥劑具敏感性，但對 strobilurins 類藥劑呈不敏感。由於 *C. siamense* 是番石榴炭疽病菌優勢族群，在藥劑的使用上應針對此類炭疽病菌，輪用不同作用機制藥劑，以降低該菌種於田間的族群數量。

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附錄 1. 植物保護資訊系統核准之番石榴炭疽病防治藥劑。

Appendix 1. Fungicides of guava anthracnose approved in plant protection information system.

Fungicide in Chinese	Fungicide in English	AI[z] (%)	FRAC[y] code	Formulation	Manufacturer
賽普護汰寧	Cyprodinil + fludioxonil	62.5	D1, E2	Water dispersible granules	Syngenta Taiwan Ltd., Taipei, Taiwan
克收欣	Kresoxim-methyl	44.2	C3	Water dispersible granules	BASF Taiwan Ltd., Taipei, Taiwan
克熱淨	Iminoctadine	40.0	M7	Wettable powder	Nippon Soda Co., Ltd., Toyama, Japan
亞托敏	Azoxystrobin	25.0	C3	Suspension concentrate	Syngenta Taiwan Ltd., Taipei, Taiwan
扶吉胺	Fluazinam	39.5	C5	Suspension concentrate	ISK Taiwan Co., Ltd., Taipei, Taiwan
得克利	Tebuconazole	25.9	G1	Emulsion, oil in water	Huikwang Co., Ltd., Tainan, Taiwan
甲基多保淨	Thiophanate-methyl	70.0	B1	Wettable powder	Ihara Chemical Co., Ltd., Tokyo, Japan
免得爛	Metiram	80.0	M3	Water dispersible granules	BASF Taiwan Ltd., Taipei, Taiwan
依普同	Iprodione	23.7	E3	Suspension concentrate	Bayer Taiwan Ltd., Taipei, Taiwan
腐絕快得寧	Thiabendazole + oxine copper	53.0	B1, M1	Wettable powder	Kuang Hwa Chemical Co., Ltd., Hsinchu, Taiwan
腈硫醌	Dithianon	42.2	M9	Suspension concentrate	BASF Taiwan Ltd., Taipei, Taiwan
鋅錳乃浦	Mancozeb	80.0	M3	Wettable powder	Huikwang Co., Ltd., Tainan, Taiwan
三氟派瑞	Trifloxystrobin + fluopyram	50.0	C3, C2	Suspension concentrate	Bayer Taiwan Ltd., Taipei, Taiwan

[z] AI: active ingridients.
[y] FRAC: Fungicide Resistance Action Committee.

附錄 2. 2019–2020 年間自番石榴園調查所收集番石榴炭疽病分離株之基因庫序列。

Appendix 2. GenBank accession numbers of *Colletotrichum isolates* collected in guava field surveys from 2019–2020.

		GenBank accession number[z]				
Species	Isolate	ITS	*GAPDH*	*TUB2*	*CAL*	*CHS-1*
C. siamense	ALCo1	MW541841	MW583606	MW583634	MW627457	MW583619
C. tropicale	ALCo2	MW541842	MW583607	MW583635	MW627458	MW583620
C. siamense	ALCo3	MW541843	MW583608	MW583636	MW627459	MW583621
C. ftructicola	DSCo1	MW541844	MW583609	-	MW627460	MW583622
C. ftructicola	SSCo1	MW541845	MW583610	-	MW627461	MW583623
C. siamense	SYCo1	MW541846	MW583611	MW583637	MW627462	MW583624
C. siamense	SYCo2	MW541847	MW583612	MW583638	MW627463	MW583625
C. siamense	SYCo3	MW541848	MW583613	MW583639	MW627464	MW583626
C. siamense	SYCo4	MW541849	MW627455	-	MW627465	MW583627
C. siamense	YCCo1	MW541850	MW583614	MW583640	MW627466	MW583628
C. ftructicola	YCCo2	MW541851	MW583615	-	MW627467	MW583629
C. siamense	YCCo3	MW541852	MW583616	MW583641	MW627468	MW583630
C. siamense	YCCo4	MW541853	MW583617	MW583642	MW627469	MW583631
C. ftructicola	YCCo5	MW541854	MW583618	-	MW627470	MW583632
C. siamense	YCCo6	MW541855	MW627456	-	MW627471	MW583633

[z] ITS: Internal transcribed spacer; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase; *TUB2*: β-tubulin 2; *CAL*: calmodulin; *CHS-1*: chitin synthase 1.

Genetic Diversity and Fungicide Sensitivity of *Colletotrichum* spp. Isolated from Guava Fields in Southern Taiwan

Shuen-Huang Tsai[1,*], Kuan-Wen Chen[2], and Chien-Jui Huang[3]

Abstract

Tsai, S. H., K. W. Chen, and C. J. Huang. 2021. Genetic diversity and fungicide sensitivity of *Colletotrichum* spp. isolated from guava fields in southern Taiwan. J. Taiwan Agric. Res. 70(2):140–156.

Guava anthracnose is one of the important diseases of guava fruit from harvest to post-harvest. The aim of this study is to understand the main species of *Colletotrichum* that currently occur in guava orchards, and to investigate the sensitivity of these *Colletotrichum* spp. to the approved fungicides registered for anthracnose management. From 2019 to 2020, symptomless leaves and diseased fruits of guava anthracnose were collected from the main production areas in southern Taiwan. Fifteen isolates were obtained and inoculated on 'Zhen-chu Ba', respectively. The results showed that wounds are needed for most isolates to cause fruit rot symptoms, and the isolates obtained from diseased fruit is generally more virulent than the isolates obtained from symptomless leaves. To identify the species of collected *Colletotrichum* isolates, five genes including internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β-tubulin 2 (*TUB2*), chitin synthase 1 (*CHS-1*) and calmodulin (*CAL*) were sequenced and a phylogenetic tree was constructed. The constructed phylogenetic tree indicated that guava anthracnose in Taiwan includes at least three species, namely, *Colletotrichum siamense*, *Colletotrichum fructicola* and *Colletotrichum tropicale*. The fungicide tests show that *C. siamense* and *C. fructicola* are generally insensitive to benzimidazoles and strobilurins, while *C. tropicale* is sensitive to benzimidazoles but it is not sensitive to strobilurins. Comparing with the other two *Colletotrichum* spp., *C. tropicale* has a different sensitivity to benzimidazoles. In addition, at concentration of 100 mg active ingredient per liter, iminoctadine and mancozeb can completely inhibit the mycelial growth of the three *Colletotrichum* spp. thiabendazole + oxine copper and dithianon can significantly inhibit the germination of the spores among three *Colletotrichum* spp. The above results can provide farmers a reference for integrated pest management of anthracnose in the field.

Key words: Guava (*Psidium guajava* L.), *Colletotrichum* spp., Phylogenetic analyses, Fungicides sensitivity test.

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